

Liver abscesses in feedlot cattle: Impact on meat quality and an alternative to antibiotic use for prevention

by

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B.S., Kansas State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

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Abstract

Liver abscesses are both a significant economic loss and animal health and welfare concern for the beef cattle feedlot industry. Currently, in-feed antibiotics are used daily throughout the finishing period to decrease prevalence and severity of liver abscesses seen at the time of slaughter. The use of antibiotics in food animal production has been under scrutiny by consumers and the associated emergence and dissemination of antibiotic resistance is of public health concern. The most common antibiotic used to prevent liver abscesses is tylosin, a macrolide antibiotic. This antibiotic class has been deemed a critically important drug class to human medicine by the FDA, and therefore tylosin is regulated by the Veterinary Feed Directive. The goal of this research was to assess the effect of liver abscesses on meat tenderness and sensory traits and evaluate the safety and efficacy of an autogenous vaccine, an antibiotic alternative, against *Fusobacterium necrophorum* at reducing liver abscess prevalence and severity in feedlot cattle. The first study assessed the Warner Bratzler Shear Force, Slice Shear Force, and meat sensory panel results for strip loin steaks in a 3×2 factorial treatment structure including variables of quality grade: Choice and Select in relation to liver abscess treatment group: normal, mild, and severely abscessed livers. The results of this study showed that liver abscess status had no effect on Warner-Bratzler Shear Force or Slice Shear Force but in sensory analysis, quality grade select steaks from cattle with mild liver abscesses were found to have greater myofibril tenderness than those with severe liver abscesses. The second study assessed the safety of an experimental autogenous liver abscess vaccine with three different proprietary adjuvant combinations, adjuvant 1 at 20% concentration, adjuvant 1 at 10% concentration, and adjuvant 2 at 15% concentration in beef cattle. All three antigen-adjuvant combinations were safe and did not elevate body temperature. The final study assessed the efficacy of an autogenous

vaccine in a commercial feedlot on cattle enrolled in a “natural” cattle program, therefore raised without antibiotics. This study found that the vaccine did not reduce prevalence or severity of liver abscesses in feedlot cattle that had been fed an average of 206 days. The research outlined in this dissertation adds to the existing body of work about the effects of liver abscesses on carcass quality and outlines the safety and efficacy of an autogenous vaccine developed with the goal of reducing liver abscess prevalence. Liver abscesses in fed cattle must be addressed to improve animal health and welfare and increase profit for producers in an already small margins industry.

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Approved by:

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Dedication

This dissertation is dedicated to my wonderful family. I couldn't have completed this work without the unwavering support of my husband, Tim McCoy. He has been by side through over 10 years of life and all of my post-secondary education, encouraging me and laughing with me through my successes and failures. My sweet daughter, Sylvia McCoy is the reason that I always strive to do better. She makes me want to leave the world a better place for the generations to come and preserve the reputation of animal agriculture so she can enjoy an industry that is so deserving of passion and is near and dear to my heart. Radar was there by my side, through every early morning and late night. My parents, John and Lisa Suhr, have always encouraged me to follow my passions and instilled in me the value of education, hard work, and a sense of humor.

Preface

Chapter 2 of this dissertation was prepared for and published in *Translational Animal Science* in 2017 (Volume 1, Issue 3, pages 304-310). Chapters 3 and 4 of this dissertation were originally prepared as final reports for the animal health company, AgriLabs, the producer of the vaccines being researched, who was acquired by Huvapharma during the execution phase of the research described in Chapter 4. These chapters have been edited to fit into a format consistent with this dissertation.

Chapter 1 - Non-antimicrobial alternatives for liver abscess prevention in feedlot cattle: A review

Introduction

Liver abscesses are a common animal welfare issue in feeder cattle and an economic loss for producers in the United States cattle feeding industry. Currently, liver abscesses are controlled with the use of in-feed antibiotics however the use of antibiotics in livestock is becoming increasingly regulated. Consumer perception surrounding antimicrobial use, particularly used as preventative measures in healthy animals, is a growing concern. It is essential to find a method to prevent liver abscesses in cattle that doesn't require the use of antibiotics and keeps beef production economically sustainable. This review will discuss various topics surrounding liver abscessation in feedlot cattle including: bacteriology, pathophysiology, economic impact, the current use of antibiotics to prevent abscesses, possible non-antibiotic prevention tools and roughage inclusion levels in feedlot diets.

Bacteriology

The most common bacterial pathogen isolated in liver abscesses in cattle is *Fusobacterium necrophorum*, sp. *necrophorum* (*F. necrophorum*). *Fusobacterium necrophorum* has been isolated from up to 100% of liver abscesses seen at the time of slaughter (Nagaraja and Chengapa, 1998; Nagaraja et al., 1999a). *Truoperella pyogenes* (*T. pyogenes*) is the second most common pathogen identified, often found in combination with *F. necrophorum* (Nagaraja et al., 1999a). Other bacteria that have been isolated from liver abscesses include *Bacteriodes* sp., *Clostridium* spp., *Pasterella* spp., *Staphylococcus* spp. and *Streptococcus* spp. (Scanlan, C.M., Hathock, 1983; Nagaraja and Lechtenberg, 2007). Interestingly, even *Salmonella enterica*, in

addition to *F. necrophorum*, has been cultured from abscesses originating from Holstein steers that had been on feed (Amachawadi and Nagaraja, 2015).

F. necrophorum is part of the normal, healthy rumen flora and increases in concentration in the rumen when cattle are transitioned to a high grain, low roughage diet such as that fed during the finishing phase (Nagaraja et al., 1999b). When the ruminal epithelium is compromised, such as occurs during ruminal acidosis, *F. necrophorum* has the opportunity to inhabit the compromised rumen wall and eventually cross the epithelial barrier, entering the bloodstream. The bacteria travels through the portal vein and causes embolic showering to the liver. In the liver, the bacteria are able to utilize virulence factors to create a favorable environment for abscess formation. This pathologic process has been termed the rumenitis-liver abscess complex (Jensen, et al., 1954).

This relationship between rumenitis and liver abscesses was first established in 1944 by H.A. Smith when a correlation was found between ruminal epithelial lesions and liver abscesses in cattle at the time of slaughter. At this time, Smith observed that of all cattle with rumen lesions, 42% had liver abscesses but only 9% of those with normal rumens had liver abscesses, and conversely, of all cattle with liver abscesses, 62% had rumen lesions. This hypothesis was further supported by Rezac et al. (2014) who noted that 32% of surveyed carcasses displaying mild or severe rumenitis also had liver abscesses but only 19% of carcasses with rumenitis had normal livers.

Fusobacterium necrophorum is a non-motile, non-spore-forming, rod to pleomorphic shaped gram-negative bacteria (Langworth, 1977). In cattle, *F. necrophorum* is associated with multiple common disease processes: liver abscesses, necrotic laryngitis (calf diphtheria), interdigital dermatitis (foot rot) and mastitis in cows (Nagaraja, et al., 2005). The bacteria uses

lactate as its major substrate, which is readily available in the rumen when cattle are on high grain rations such as most feedlot diets. High grain fed cattle have at least a 10-fold increase in *F. necrophorum* when compared to forage fed cattle (Berg and Scanlan, 1981). The bacteria normally reside in the rumen fluid, but can also be found adhered to the rumen wall (Narayanan, et al., 1998). When the rumen wall is compromised, it allows the bacteria to infect the damaged epithelium, causing rumenitis. This can happen in the case of ruminal acidosis, which is most commonly associated with liver abscesses, but it has also been hypothesized that other forms of rumen epithelial damage can include trauma from ingested foreign material, abrasive feedstuffs or even hair from self-grooming causing microabrasions (Fell et al., 1972).

Fusobacterium necrophorum has a unique synergistic relationship with *T. pyogenes*, a gram-positive bacterium which is also a normal inhabitant of the rumen. *Trueperella pyogenes* is a facultative anaerobe and is often isolated in liver abscesses in addition to *F. necrophorum*, occurring in 2-20% of all abscesses where *F. necrophorum* is the primary agent (Nagaraja, et al., 1996). Nagaraja et al., (1999a) found that *T. pyogenes* is more prevalent in abscesses isolated from cattle that received tylosin during the feeding period than those that did not (53 vs 10%), however the cause of this remains unknown.

Fusobacterium necrophorum virulence factors include leukotoxin, endotoxin (LPS), and hemagglutinin (Tadepalli, et al., 2009). Leukotoxin appears to be the most important virulence factor, causing killing of bovine peripheral leukocytes through cellular activation and apoptosis in low concentrations and necrosis at high concentrations (Tan, et al., 1994, Narayanan et al., 2002). The strains of *F. necrophorum* isolated in liver abscesses are more leukotoxic than those strains isolated from the rumen wall, implying that highly leukotoxic strains have an advantage in abscess formation (Tan, et al., 1994). Leukotoxin production has also been correlated to the

abscess formation in laboratory animals (Coyle-Dennis and Lauerman, 1979). *Trueperella pyogenes* is able to use Oxygen in the liver, creating an anaerobic environment for the *F. necrophorum* to thrive (Takeuchi, et al., 1983). Lactate, the primary substrate of *F. necrophorum* is an end product of *T. pyogenes* metabolism (Takeuchi, et al., 1983). They synergize by the virulence factors of *F. necrophorum* providing protection from phagocytosis to *T. pyogenes*, and *T. pyogenes* providing the substrate and anaerobic environment needed by *F. necrophorum*. A capsule is formed around the abscess, walling off the bacteria from the host's defense system, allowing a more favorable environment for all bacteria (Lechtenberg, et al., 1988). Histologically, inside of the capsule, these abscesses are pyogranulomatous, containing an inflammatory area surrounding a necrotic core (Lechtenberg, et al., 1988).

Abscess Grading and Carcass Effects

A modified Elanco liver abscess grading system (Elanco, Greenfield, IN) is commonly used to classify abscess severity. Often a 3 category system is used with classes being normal, A and A+. A common modification of this system is often used in research that adds an additional classification of A- for mild abscessation (Rezac et al., 2014). Normal livers are free of abscesses, scars, liver flukes, or any other pathology. Livers classified as A- have ≤ 2 abscesses and each abscess present is ≤ 2 cm in diameter. Livers showing resolved abscess scars but without active abscesses are also often classified as A-. Livers scored as A have 2 to 4 abscesses, each 2 to 4 cm in diameter. A+ livers have ≥ 4 abscesses or at least one abscesses that is ≥ 4 cm in diameter. Additional classifications often added include A+AD, when the liver is adhered to the diaphragm and/or body wall, and A+OP, where the abscesses are open, possibly contaminating other organs causing condemnation.

Liver abscess prevalence can vary widely, ranging from 0 to 80% in different studies (Amachawadi and Nagaraja, 2016). Many factors influence the prevalence of liver abscesses in a group including cattle factors, environmental factors, and management factors (Reinhardt and Hubbert, 2015, Amachawadi and Nagaraja, 2016). Liver abscess prevalence is slightly greater in steers than heifers (often 1 to 3% greater), which is believed to be due to a slightly elevated feed intake and the fact that heifers often mature earlier than steers, resulting in fewer required days on feed (Hicks, et al., 1990; Nagaraja and Lechtenberg, 2007). Holsteins have a much greater prevalence than beef cattle (often more than double the prevalence), and it is speculated that this is due to significantly increased time on feed and an elevated daily feed intake (Hicks, et al., 1990; Nagaraja and Lechtenberg, 2007; Amachawadi and Nagaraja, 2016). Grooming behavior may also contribute to liver abscess prevalence, as Holsteins have been observed to groom themselves and penmates more than beef cattle (Fell, et al., 1972). Liver abscesses are seen most commonly in the Plains states than other regions of the US including the Northwest, Southwest, and Midwest (Reinhardt and Hubbert, 2015). The reason for this geographic difference is unknown but is speculated to be affected by ration differences and possibly different strains of *F. necrophorum* endemic to different soil types. In fact, the majority of feedlots in regions where cattle have lower liver abscess rates (those in the Northwest, Southwest, and Midwest) do not need to include tylosin in the diet of their finishing cattle.

The 2016 National Beef Quality Audit recorded 17.8% of the fed cattle observed at abattoirs had liver abscesses, contributing to a 30.8% liver condemnation rate (Eastwood, et al., 2017). Other common liver defects seen at slaughter include liver flukes (also referred to as distoma), cirrhosis and telangiectasis. Livers are commonly condemned due to contamination at slaughter, this can be from a ruptured liver abscess or condemnation from other organs such as

rumen contents. This percentage of liver abscesses in fed cattle is increased when compared to the last 15 years of Beef Quality Audits. In 2010, 13.7% of observed livers contained abscessation, in 2005, 13.4%, and in 2000, 13.6% (McKenna, et al., 2002; Garcia, et al., 2008, McKeith, et al., 2012). This increase could be due to an increase in the percentage of cattle on “natural” programs which restrict the use of hormones and antibiotics during the finishing period, so these cattle never receive tylosin.

Liver abscesses can cause significant economic loss during the finishing period and at slaughter (Brink, et al., 1990; Brown and Lawrence, 2010). Brink, et al. (1990) analyzed data from 12 different experiments in which animals were individually fed. Variables that differed between normal livers and those with A+ scored livers included end weights, HCW, dressing percentage and feed intake, all tending to be less for steers with abscessed livers than their counterparts with normal appearing livers. These losses include decreased HCW, ribeye area, marbling scores for cattle (Brown and Lawrence, 2010). Brown and Lawrence (2010) also found that the greatest economic losses associated with liver abscesses are those carcasses with livers scoring A+, especially those livers that are adhered to the diaphragm, abdominal organs, or body cavity. When the abscesses are adhered to the body cavity, these areas have to be cut away from the carcass, increasing trim loss. This estimated trim loss ranges from 3 to 13.2 kg in different studies (Davis et al., 2007; Brown and Lawrence, 2010). It has been estimated that severe liver abscesses can decrease the value of an affected carcass by \$38 (Brown and Lawrence, 2010), to more than \$52 (Reinhardt and Hubbert, 2015), however due to cattle market fluctuations, this value could be even higher today.

While the effects of liver abscesses on feedlot performance and carcass characteristics has been well documented, the effects of liver abscesses on meat tenderness within quality grade

had not been investigated before this author's study (McCoy et al., 2017). Animal health during the finishing phase can greatly worsen feedlot performance, decrease both quality and yield grade, and even decrease meat tenderness within quality grade (Gardner et al., 1999). Gardner et al., (1999) fed cattle in a commercial feedlot for 150 days and monitored cattle for clinical signs of respiratory disease daily and lung lesions at the abattoir. Shear force values were analyzed on Longissimus steaks and were found to be lower in steaks from cattle without lung lesions when compared to those with active lesions at the time of slaughter (3.6 vs 4.0 kg). McCoy et al., (2017) investigated the effects of liver abscess severity on meat tenderness assays and sensory attributes in cattle finished at a commercial feedlot on a diet that did not contain tylosin phosphate. This study reported that liver abscess status had no effect on Warner-Bratzler Shear Force or Slice Shear Force but in sensory analysis, quality grade Select steaks from cattle with moderate liver abscesses were found to have greater myofibril tenderness than those with severe liver abscesses ($P < 0.03$). The results of this study indicate that liver abscesses do not have an impact on meat tenderness as measured by WBSF or SSF and do not impact beef juiciness or flavor attributes but may impact the myofibril tenderness of quality grade Select beef. This was an important finding as the beef industry may soon be faced with the task of reducing antibiotic use, specifically tylosin, and in turn, increasing liver abscesses in our fed cattle population if an effective alternative prevention technique is not identified soon. If this occurs, McCoy et al. demonstrated that consumers will not have a decreased eating experience due to liver abscesses other than the decrease in quality grade, which consumers are aware of when making a purchasing decision.

Tylosin

Macrolide antibiotics such as tylosin phosphate have proven effective at decreasing the concentration of *F. necrophorum* in the rumen and in turn decreasing liver abscesses. Nagaraja, et al. (1999b) showed a decrease in ruminal *F. necrophorum* levels when adding tylosin phosphate at the approved label dose (90 mg/hd/d) to an 80% concentrate diet in ruminally cannulated animals. This study found that feeding tylosin had no effect on ruminal fermentation products including pH, VFAs, lactate, or ammonia. Continuous feeding of tylosin did reduce *F. necrophorum* levels further over time during the 31 day feeding period suggesting continued susceptibility to the antibiotic and results suggested that the number of resistant bacteria did not increase over time.

Tylosin has been shown to reduce the prevalence of liver abscesses by up to 81.8% in large pen studies (Brown, et al., 1973, Brown et al, 1975). Both tylosin phosphate and tylosin urea adduct have been shown to be effective at reducing liver abscesses however Tylosin, the macrolide on the market today labeled for reduction of liver abscesses is in the form of tylosin phosphate (Brown, et al., 1973). Both forms of tylosin were found to reduce the incidence of liver abscesses and in turn increase average daily gain, and improve feed conversion in the initial study by Eli Lilly and Company (Brown, et al., 1973). Brown et al. (1975) compared tylosin and chlortetracycline and found that both reduced the incidence of liver abscesses compared to control cattle, however tylosin produced a greater reduction in abscesses.

Nagaraja et al., (1999a) cultured *F. necrophorum* and *T. pyogenes* from liver abscesses originating from cattle that had received tylosin and a group that had not. This study found no difference between the isolates from these two groups for the MIC of tylosin, chlortetracycline, oxytetracycline, tilmicosin, virginiamycin, bacitracin, lasalocid, or monenin. These results

indicate that the use of tylosin does not cause resistance of these 2 species of bacteria to the tested antimicrobial compounds. However, this study did not investigate the effects of daily tylosin administration on antibiotic resistance in other species of bacteria in the rumen.

In a small pen study, Muller et al. (2018) intermittently fed tylosin, feeding it off label at 1 week on, 2 weeks off and compared it to cattle fed without tylosin, and a group fed tylosin continuously during the finishing period (119 days). There was no difference in liver abscess prevalence between the group receiving continuous and intermittent tylosin (7.84 vs 9.62%), however they both differed from the group receiving no tylosin (21.36%). Muller et al. (2019) also concluded that *Enterococcus* bacterial counts didn't differ between groups but all groups developed macrolide resistance in the *Enterococcus* bacteria over time. These authors concluded that macrolide resistance develops not because of macrolide administration to the cattle of interest but instead is acquired through the environment that has developed due to long term antibiotic usage. Results from this study indicate that intermittent feeding of tylosin can reduce the overall total use of antimicrobials by feedlots without compromising liver abscess prevention and will not likely cause additional macrolide antibiotic resistance issues over those caused by daily tylosin administration.

Regulatory Concerns

A concern with antimicrobial use in food animals is the potential contribution to antimicrobial resistance in both veterinary and human medicine. Macrolide antibiotics, the class of antibiotics to which tylosin belongs, are considered a critically important drug class in human medicine (FDA, 2003). In 2012, the U.S. Food and Drug Administration (FDA) issued guidance 209, a document intended to provide guidance to the food animal industry on the use of medically important antibiotics to human medicine and their use in food-producing animals. This

document outlined two main principles including limiting the use of medically important antimicrobial drugs in food animals to those necessary to assure animal health, therefore discouraging the use of these drugs for growth promotion purposes, and ensuring that these medications are only used in food animals under the direction of a veterinarian. In 2013, guidance 213 was published, re-emphasizing the information in guidance 209 and outlining a timeline for the transition of these drugs from over-the-counter status to prescription or Veterinary Feed Directive Status. At this time, it was also requested of the food animal industry to voluntarily phase out medically important antimicrobials being used for production purposes. The Veterinary Feed Directive (VFD) final rule came into effect in 2017 and at this time it was federally mandated that all medically important antibiotics administered through food and water to food producing animals had to be under the written supervision of a veterinarian in accordance with all label directions, which could no longer include any growth promotion claims. For the purpose of this paper, these documents federally regulated tylosin, mandating that it can only be administered according to label directions, including dosages, under the written direction of a veterinarian.

There is some speculation that antimicrobials considered medically important to human medicine will become increasingly regulated in food animal species and this could increase liver abscess incidence in feedlot cattle. The industry is actively searching for an alternative solution to the use of daily in-feed antibiotics to prevent this animal welfare and economic concern. Below is discussion of methods of non-antibiotic liver abscess prevention that have been researched including vaccination against the causative agents, feed additives such as essential oils, and a brief review of the impact of diet on liver abscesses.

Vaccines

Vaccination against *F. necrophorum* and *T. pyogenes* has been a widely researched as a liver abscess prevention technique. If a vaccine was available that was as efficacious as tylosin, feedlot antimicrobial use would be significantly reduced and it could be competitively priced when compared to daily tylosin administration. Vaccines are also allowed to be administered in cattle fed as part of “natural” programs, a market that currently has no commercially available liver abscess prevention tools.

Leukotoxin has been heavily investigated as a potential vaccine component (Tan et al., 1994b; Sanginala, et al., 1996; Sanginala et al., 1997). The virulence of leukotoxin combined with the high molecular weight (>300,000) of the protein make it a highly immunogenic compound (Narayanan, et al., 2002). Anti-leukotoxin antibody titers have been shown to correlate to severity of liver abscesses, those animals with higher antibody titers producing less severe abscesses (Tan et al., 1994). Sanginala et al. (1996) developed experimental vaccines against *F. necrophorum* subsp. *necrophorum* with 3 different antigen types including a inactivated whole-cell culture, a crude leukotoxin, and a semipurified leukotoxin used in combination with Saponin and oil emulsion adjuvants. Cattle were inoculated intraportally with *F. necrophorum* and serial serum antibody levels were measured. The culture supernatant produced increased titers of serum leukotoxin-neutralizing antibody, IgG and IgM in vaccinated cattle. As a follow up to this study, Sanginala et al. (1997) investigated this vaccine containing a cell-free culture supernatant of a high leukotoxin-producing strain of *F. necrophorum* that had been inactivated combined with an adjuvant. This product was used in 3 different doses (1, 2, and 5 mL) with 2 doses being administered, 21 days apart. Twenty one days following the revaccination, the *F. necrophorum* culture was injected intraportally to experimentally induce liver abscesses. Antileukotoxin

antibody titers did develop in a dose-dependent fashion and were found to be higher in steers that did not develop abscesses when compared to those that did. The results of this study indicated that vaccination against *F. necrophorum* using a leukotoxin antigen could reduce liver abscess incidence and severity.

Jones et al., (2004) conducted two studies at a commercial feedlot investigating a *T. pyogenes* - *F. necrophorum* bivalent bacterin-toxoid vaccine administered as a single dose product. The vaccine contained an inactivated *F. necrophorum* leukotoxin and *T. pyogenes* pyolysin that was used in combination with a water-in-oil adjuvant. Two antigen levels were used, and the high antigen containing vaccine was found to be effective, reduce liver abscess prevalence by 37.5 to 48.4% (16% vaccinates vs 31% controls in study one and 30% vaccinates vs 48% controls in study two) in cattle on a high grain ration. This product was later commercially available as Centurion, first produced by Schering-Plough Corp, now Merck Animal Health (Omaha, NE). Centurion was labeled to “aid in the reduction of liver abscesses” and is no longer commercially available.

Fusogard (Elanco Animal Health US, Inc, Greenfield, IN) is a *F. necrophorum* bacterin vaccine labeled to “aid in the reduction of clinical signs of footrot and the number and size of liver abscesses caused by *F. necrophorum*”. This product is still currently on the market, and used in some feedlots, primarily due to the footrot prevention benefits. Fusogard has been shown to be effective in groups of cattle with low overall prevalence of liver abscesses (decreasing overall abscesses from 10% to 2%), but is less effective in groups of cattle with an increased overall prevalence (decreasing abscesses from 30% to 24%) (Checkley, et al., 2005). In a comparative study of Centurion and Fusogard with a negative control, Fox et al. (2009) found that neither vaccine reduced liver abscess prevalence nor severity in a group of cattle that had

high liver abscesses prevalence (56% total abscess prevalence, 39% severe). This author conducted research using an autogenous vaccine against *F. necrophorum* in an attempt to reduce the prevalence of liver abscesses seen at slaughter, however this vaccine was not found to reduce liver abscesses in natural fed cattle in a large pen trial. This data can be found in chapter 3 of this dissertation.

To summarize these findings, vaccination may be sufficient as a prevention approach in groups of cattle with low liver abscess incidence or when used in combination with other management strategies and prevention methods. Currently there are no vaccines on the market that are effective at reducing liver abscess prevalence or severity in groups of cattle with high prevalence. This may change in the future as this is an area where many research efforts have been focused.

Feed Additives

Essential oils have been used experimentally attempting to reduce liver abscesses by reducing the amount of *F. necrophorum* in the rumen. Essential oils are natural chemical compounds derived from plants that have been used for a variety of purposes, including their potential as antimicrobials. The antimicrobial properties of essential oils have been widely studied and their mechanism of action is attributed to the disintegration of the bacterial cell membrane (Helander, et al., 1998; Hammer, et al., 1999). In vitro, limonene, at 20 or 100 µg/mL, and thymol 100 µg/mL inhibit *F. necrophorum* growth, however eugenol, guaiacol, and vanilla did not inhibit *F. necrophorum* growth (Elwakeel, et al., 2013). In contrast, Samii., et al. (2016) found that both limonene linear and quadratically reduced *F. necrophorum* growth in-vitro at concentrations up to 100 µg/mL but did not completely inhibit growth as previously found. This study also found that thymol linearly reduced *F. necrophorum* growth in-vitro at concentrations

up to 100 µg/mL but showed less of an effect than limonene. Samii, et al. (2016) also noted that there was no synergistic effect between limonene and thymol in-vitro.

In ruminally cannulated animals limonene decreased ruminal concentrations of *F. necrophorum* (Samii, et al., 2016). This study found that 40 mg/kg of limonene was most effect at decreasing *F. necrophorum* concentrations, decreasing from 2.65 log₁₀ in the control animals to 2.15 log₁₀ in the 40 mg/kg treatment group. The authors acknowledged that although a decrease in ruminal *F. necrophorum* concentrations occurred, this may not reduce liver abscess prevalence due to the relatively small magnitude of reduction and the killing of *F. necrophorum* in the rumen fluid versus those adhered to the rumen epithelium. The effects of thymol was not investigated in-vivo in this study.

Active yeast products have been used in feedlot rations and are proposed to impact rumen health and in turn liver abscesses. Ran et al. (2018) investigated supplementing a finishing diet with an active dried yeast product in two forms- ruminally protected and non-protected. Non ruminally protected active dried yeast and a mixture of the protected and non-protected active dried yeast products both decreased severely abscessed livers seen at slaughter compared to those cattle receiving no antibiotics, those receiving monensin and tylosin, and those receiving only a protected active dried yeast product. Overall liver abscess prevalence did not differ between the treatment groups. These results imply that the yeast works at the level of the rumen, possibly altering rumen microflora. Interestingly, cattle receiving the yeast product in either form also tended to have lower fecal *Escherichia coli* counts compared to both the control group and the group receiving tylosin and monensin.

Roughage

Roughage levels in the finishing ration is assumed to play a key role in liver abscess formation due to its influence on rumen pH, subsequent acidosis and rumenitis. Increasing roughage during the finishing period has proven effective at decreasing the prevalence of liver abscesses in rations containing many different grain and roughage sources (Brent, 1976; Zinn and Plascencia, 1996; Loerch and Fluharty, 1998). This is a vast area of research in ruminant nutrition and will only be briefly discussed here.

Zinn and Plascencia (1996) found that increasing alfalfa level in a feedlot ration from 10 to 30% decreased liver abscess prevalence by 86% at the time of slaughter following a 135 day feeding period (15.2 vs 2.1%). Loerch and Fluharty (1998) added corn silage as a roughage source to a concentrate diet for the last 102 days of feeding period with treatment groups receiving a 100% concentrate diet or a group receiving an 85% concentrate and 15% corn silage. Those cattle receiving 15% roughage had fewer abscessed livers seen at the time of slaughter than those cattle receiving only concentrate (14.7% vs 29.3%).

Bartle et al. (1994) showed that increasing roughage from 2% to 10% in 2 different rations, one containing steam-flaked sorghum and one with whole-shelled corn for the last 28 days of the finishing period can reduce liver abscess prevalence (9% vs 0% in the sorghum diet and 6% vs 0% with whole-shelled corn diet). It is proposed that this allows time for the liver abscesses that have formed during the finishing period to heal before the cattle are sent to slaughter. Loerch and Fluharty (1998) found that cattle receiving a 100% concentrate diet for the first 84 days but were then switched to a 85% concentrate, 15% roughage (corn silage) diet for the last 102 days of the feeding period did not differ in liver abscess prevalence at slaughter compared to those that were receiving 15% roughage for the entire 186 day feeding period. Both

of these groups had lower liver abscesses when compared to treatment groups receiving 100% concentrate rations for the entire feeding period, or only the last 102 days of the feeding period. Those cattle receiving a 100% concentrate ration for the entire 186 day feeding period did have a higher overall gain/feed ratio than those cattle receiving a 85% concentrate for the entire period (0.192 vs 0.174 kg), however neither of these groups differed statistically for this variable from either of the two groups, those switched from the 100% ration to the 85% ration on day 84 or the group that switched from the 85% to 100% ration on day 84. Reinhardt and Hubbert (2015) state that roughage is a costly ration ingredient on a per-unit-of-net-energy basis and can be costly and difficult to handle. Increasing roughage during the end of the feeding period would mitigate these issues, however animal welfare concerns are still applicable as cattle would still have the opportunity to form abscesses early in the feeding period and would just be given an opportunity to heal.

Interestingly, not only roughage level, but also the form of roughage in the ration appears to have an impact on the prevalence of liver abscesses. Calderon-Cortes and Zinn (1996) fed a steam-flaked corn based diet with a roughage source of alfalfa hay chopped at 2 stem lengths and fed at 3 different inclusion rates, 0, 8, and 16%. This study found that fiber coarseness, and not roughage level influenced liver abscesses with finer alfalfa producing 12.5% abscesses vs 0% abscesses with courser alfalfa in the 8% alfalfa ration. Utley et al. (1973) found that cattle fed a ground corn based diet with 20% whole peanut hulls had fewer liver abscesses than if these peanut hulls were ground or pelleted (4% vs 52% and 59%). These results indicate that the stimulation of the roughage on the rumen wall may impact rumen health and decrease liver abscess formation and that roughage inclusion is not only beneficial due to the energy dilution effects.

Conclusion

Liver abscesses continue to be a persistent problem for the US cattle feeding industry and may even increase in concern as the federal regulation of antimicrobial use in food producing animals increases. While tylosin phosphate has been successful at reducing liver abscesses, alternatives are actively being researched to help reduce antibacterial use in food animals. The causative against *F. necrophorum*, which synergizes with *T. pyogenes*, has been a target for vaccine development for many years. Vaccination has been shown to be successful at liver abscess prevalence and severity reduction in cattle with relatively low prevalence of liver abscesses. Therefore, vaccination used in combination with other methods to reduce liver abscesses but does not decrease liver abscess prevalence in groups of cattle with high prevalence. This dissertation contains research on a new vaccine, which is an autogenous vaccine against *F. necrophorum* including a novel adjuvant that had been successfully used in other vaccines. It is possible that vaccination in combination with increased roughage levels or a change in roughage source could have an increased reduction in liver abscesses, challenging the current reduction levels seen with tylosin, therefore negating the need for antibiotics to prevent this disease. Essential oil compounds, particularly limonene show potential to reduce liver abscesses when included in feedlot rations, but more research is needed on this topic. Active yeast products may reduce severity of abscesses but have not been shown to decrease overall liver abscess prevalence. Increased roughage in the ration, even if only for the last 28 days of the feeding period and the feeding of course roughage sources can decrease abscesses seen at the time of slaughter. Liver abscesses are truly a disease at the junction of animal health and nutrition. The veterinary and nutrition communities must work together to find an alternative to antibiotic use to decrease liver abscesses in the feedlot industry moving forward.

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Chapter 2 - Effects of liver abscess severity and quality grade on meat tenderness and sensory attributes in commercially finished beef cattle fed without tylosin phosphate

Abstract

Strip loin steaks (n = 119) were used to evaluate the effects of liver abscess severity and USDA quality grade on meat tenderness and sensory attributes of steaks from finished feedlot cattle. Steaks were used in a 3×2 factorial treatment structure using a completely randomized design and were collected at a commercial abattoir located in northwest Texas. All cattle were sourced from a single feedlot and fed a common diet that did not include tylosin phosphate. Treatments were USDA quality grades of Select (S) and Low Choice (C) and liver abscess scores of normal (N; healthy liver with no abscesses), mild (M; 1 abscess less than 2 cm in diameter to 4 abscesses less than 4 cm in diameter), and severe (SV; 1 abscess greater than 4 cm in diameter or greater than 4 small abscesses). All steak samples were collected on the same day and were cut from the left side of the carcass at the 13th-rib by a trained abattoir employee. Steaks were vacuum-packaged, and aged at 3 ± 1 °C for 14-d post-mortem. Warner-Bratzler Shear Force (WBSF) and Slice Shear Force (SSF) analyses were conducted and cook-loss percentage was measured. A trained sensory panel analyzed samples for juiciness, tenderness, and flavor attributes. There were no differences among liver abscess scores for WBSF or SSF ($P > 0.52$). Warner-Bratzler Shear Force was lower for C-SV than S-SV ($P = 0.04$). Sensory attributes of initial and sustained juiciness, and overall tenderness were all greater for C than for S steaks ($P < 0.04$) and connective tissue amount was less for C steaks when compared to S ($P = 0.03$). Liver abscess score had no effect on any sensory attributes ($P > 0.70$); however, there was

an interaction between quality grade and liver score for myofibillar tenderness ($P = 0.03$). Within C steaks, liver abscess score had no effect on myofibrillar tenderness ($P > 0.05$), however, in S steaks, M steaks were more tender than SV steaks ($P < 0.03$).

These results indicate that within quality grades, meat tenderness or sensory attributes were not influenced by liver abscess score but that M liver abscesses may affect the myofibrillar tenderness of S steaks.

Key words: cattle, feedlot, liver abscesses, meat tenderness, palatability, quality grade

Introduction

Liver abscesses are a significant problem in the United States' cattle feeding industry, costing the industry an estimated \$15.9 million annually in liver condemnation, trim losses, and reduced carcass weights and quality grades (Brown and Lawrence, 2010; Hicks, 2011). The 2011 National Beef Quality Audit reported the average prevalence of liver abscesses in cattle surveyed was 13.7% (McKeith et al., 2012) with incidence rates ranging from 10 to 20% in recent literature (Amachawadi and Nagaraja, 2016). Previous reviews have reported liver abscess incidence is influenced by a number of factors including: breed, gender, diet, days on feed, cattle type, season, and geographical location (Nagaraja et al., 1996; Reinhardt and Hubbert, 2015).

Liver abscesses occur subsequently to rumen insults caused by acidosis and rumenitis, often referred to as the “rumenitis-liver abscess complex” (Jensen et al., 1954). Cattle fed readily-fermentable concentrate-based diets often have lower rumen pH levels that have the potential to cause damage to ruminal epithelium tissue (Haskins et al., 1969). It has been proposed that pathogens associated with liver abscess formation enter the blood stream through perforated rumen epithelium and are transported to the liver through the portal vein (Smith, 1944; Nagaraja and Chengappa, 1998).

Brown and Lawrence (2010) showed a reduction in HCW, dressing percentage, yield grade, LM area, and marbling scores for carcasses with multiple large abscesses and decreased marbling scores for severe liver abscesses with adhesions when compared to their counterparts with normal livers. Although the effect on carcass characteristics has been researched, no previous work has evaluated the effect of liver abscess status on meat tenderness and sensory attributes. Therefore the objective of this study was to determine the effects of liver abscess severity on meat tenderness and sensory attributes of steaks from USDA Low Choice and Select quality grades.

Materials and methods

Institutional Animal Care and Use Committee approval was not required for this study because no live animals were handled. The protocols for the use of human subjects for this study were reviewed and approved by the Kansas State University Institutional Review Board (IRB #7440).

Carcasses:

For this study, Bos Taurus steers originated from the same commercial feedlot and were fed common diets that did not contain tylosin phosphate. All carcasses utilized in this study were from cattle that were slaughtered on a single day at a commercial abattoir in northwest Texas and carcasses were selected after lungs and livers were scored. Only carcasses with healthy, normal lung scores were utilized to avoid any potential effects on tenderness or sensory attributes caused by respiratory disease.

Liver Scores:

Liver scores were evaluated and recorded by trained observers at harvest using the scoring system previously defined by Rezac, et al. (2014). Livers were scored as 0 (no

abscesses); A- (1 to 2 abscesses less than 2 cm in diameter); A (2 to 4 abscesses between 2 and 4 cm in diameter); A+ (1 abscess greater than 4 cm in diameter or greater than 4 small abscesses); and A+/AD (A+ criteria with adhesions to the body cavity; Rezac et al., 2014). For this study, 0 represented the normal (N) liver population, A- and A represented the mild (M) liver abscess population, and A+ and A+ with adhesions represented the severe (SV) liver abscess population. At the time of liver scoring, carcasses were given a sequence number that was then correlated back to the plant identification number.

Steak Collection:

Quality grades of USDA Low Choice (C) and Select (S) and liver abscess scores of N, M, and SV were used for this study. Carcasses were chilled at the abattoir for approximately 36 h postmortem before grading. USDA quality grades were assigned to carcasses at the grading stand and carcass tags were used to identify carcasses in each treatment category. Colored tags were placed onto the carcasses of interest and they were railed off for steaks to be collected. Strip loin steaks were cut approximately 6.35 cm thick from the left side of the carcass at the 13th rib by a trained abattoir employee the same day they were graded. Steaks from a total of 119 carcasses were collected and consisted of the following: 22 C-N; 20 C-M; 20 C-SV; 21 S-N; 20 S-M; and 16 S-SV. Steaks were put into labeled Ziploc freezer bags and put on ice to be transported back to Manhattan, KS, where steaks were vacuum-packaged and aged at $3 \pm 1^{\circ}\text{C}$ for 14 d postmortem. Steaks were stored for 48 h at -20°C then faced and cut into two 2.54 cm steaks using a band saw (BIRO Model 3334; BIRO Manufacturing Company, Marblehead, OH). The more anterior steak was used for sensory panel and the more posterior steak was used for instrumental tenderness measures.

Shear Force Measurements:

Laboratory assays were conducted at the Kansas State University Meat Laboratory (Manhattan, Kansas). Warner-Bratzler Shear Force (WBSF) and Slice Shear Force (SSF) were conducted according to the American Meat Science Association (AMSA) Research Guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat (AMSA, 2015). Steaks were randomized using a random number generator and thawed for 24 h at 3° C. Steaks were weighed before and after cooking to calculate cook-loss. Before cooking, a 30 gauge copper/constantan thermocouple was inserted into the geometric center of each steak. Steaks were cooked on clamshell grills (Cuisinart Griddler, East Windsor, NJ) set to 176.7° C that had been sprayed with nonstick cooking spray. Internal temperatures were monitored with a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Steaks were removed from the grill when an internal temperature of 65.6° C was reached for a target endpoint temperature of 70° C. Once maximum rise in temperature was reached, thermocouples were removed and steaks were cut for SSF using a SSF kit (G-R Elec. Mfg., Manhattan, KS). An Instron testing machine (Model 5569; Instron, Canton, MA) was used in combination with a SSF blade (crosshead speed of 500 mm/min). After SSF, the remaining portion of the steak was cooled overnight at 3° C and used for WBSF. Six 1.27 cm cores, parallel to the muscle fiber orientation, were removed and sheared on the Instron testing machine with a v-blade (crosshead speed of 250 mm/min) for WBSF analysis. The values of the 6 cores were averaged to obtain a single WBSF value for each steak.

Sensory Analysis:

Panelists were trained over a series of training sessions with a minimum number of 3 trainings attended over a 5 d period with a 2 d period between the end of trainings and the beginning of panels. Characteristics on which panelists were trained included initial juiciness,

sustained juiciness, myofibrillar tenderness, connective tissue, overall tenderness, beef flavor intensity, and off-flavor intensity. Anchors described by Adhikara et al. (2011) were provided at each training session and were used to set the 100 point beef flavor intensity scale. Sensory scales ranged from 0 to 100 with 0 being extremely dry, tough, none, or bland, 50 being neither dry nor juicy, neither tough nor tender, and 100 being extremely juicy, tender, abundant, or intense.

Steak samples for sensory analysis were stratified by liver score and USDA quality grade and randomly assigned to one of 20 sensory panels so that each panel had one steak from each treatment combination. Six samples were evaluated per panel with a maximum of two panels per day. Steaks were prepared in the same manner as described above for WBSF and SSF.

Immediately after peak temperature was reached, steaks were cut into uniform $1.3 \times 1.3 \times 2.5$ cm cubes using a Sensory Evaluation Box (G-R Elec. Mfg., Manhattan, KS) and placed into a metal double boiler to remain warm until served. Panels consisted of 20 sessions with 7 to 9 trained panelists per session. Panelists were seated in individual sensory analysis booths lit with low-intensity red and green incandescent light to mask any color differences. Unsalted crackers, apples, and deionized, distilled water were provided as palette cleansers. Digital tablet computers were used to record sensory data on each steak with each category having a continuous line scale from 0 to 100. Qualtrics analytics software (Qualtrics 2015, Version 2417833, Provo, UT) was used to record and summarize data.

Statistical Analysis:

Sensory panel, WBSF, and SSF data were analyzed using the GLIMMIX procedures of SAS (version 9.4; SAS Inst. Inc., Carry, NC). Sensory panel data were averaged within each steak and averages were used for analysis. Quality grade, liver score, and their interaction were

analyzed as fixed effects and panel number was used as a random effect. Warner-Bratzler Shear Force and SSF data were analyzed with quality grade, liver score, and their interaction as fixed effects, and off peak temperature used as a covariate. A Kenward-Roger adjustment was applied to the degrees of freedom for all analyses. Significance was determined at $P < 0.05$.

Results

Sensory Panel:

Results for sensory panel data can be found in Table 2.1. There were no interactions for any combination of grade \times liver score for initial juiciness, sustained juiciness, connective tissue amount, overall tenderness, beef flavor intensity, off flavor intensity or cook loss ($P \geq 0.06$).

There was a quality grade effect for initial juiciness, sustained juiciness, connective tissue amount, overall tenderness, and beef flavor intensity ($P < 0.05$). As expected, C steaks were greater ($P < 0.02$) for initial and sustained juiciness compared to S. Liver abscess score had no effect ($P > 0.70$) on initial or sustained juiciness.

There was a quality grade \times liver score interaction for myofibrillar tenderness ($P = 0.03$). Within C steaks, liver abscess score had no effect on myofibrillar tenderness ($P > 0.05$), however, within S steaks, M steaks were more tender than SE steaks ($P < 0.03$; Figure 2.1).

Select steaks had a greater ($P < 0.04$) amount of connective tissue than C. Liver abscess status had no effect ($P > 0.70$) on connective tissue amount among N, M, or SV treatments. Liver abscess score had no effect ($P = 0.79$) on overall tenderness.

For beef flavor intensity and off flavor intensity, neither quality grade nor liver abscesses scores differed ($P > 0.26$).

Warner-Bratzler Shear Force and Slice Shear Force

Warner-Bratzler Shear Force and SSF data is presented in Table 2.1. There was no quality grade \times liver score interaction for WBSF values ($P = 0.38$). Neither quality grade nor liver score affected ($P > 0.08$) WBSF values. There was no interaction for quality grade \times liver score for SSF values ($P = 0.61$). Slice Shear Force was not affected by quality grade or liver score ($P > 0.38$). Cook loss was not affected ($P > 0.20$) by quality grade or liver score.

Discussion

Liver abscesses are a concern in the beef industry as an economic loss to feedlots and abattoirs in addition to a possible animal welfare concern, specifically with severe abscesses (Reinhardt and Hubbert, 2015). Liver abscesses can decrease value of beef carcasses by up to \$38 per animal (Brown and Lawrence, 2010). All liver abnormalities are estimated to cost the industry more than \$15 million annually in lost liver value alone, with 67% of these abnormalities being liver abscesses (Brown and Lawrence, 2010). According to the 2011 National Beef Quality Audit liver condemnation had an incidence rate of 20.9% with 5.4% being condemned for major abscesses, 8.3% due to minor abscesses, and the remainder being for flukes, contamination, or other reasons (McKeith et al., 2013). Fortunately, results from this study indicate that liver abscesses do not effect meat tenderness or sensory attributes within C and S quality grades.

Carcasses selected for this study were selected to have normal lungs with no consolidation. When liver abscesses rupture at the abattoir, all offal affected is condemned. When this occurred during this study's data collection, lungs were not able to be scored and therefore these carcasses were not included in this study. Consequently, a portion of the carcass population with SV liver abscesses were not eligible for this study.

Product consistency is important to ensure consumer satisfaction, therefore it is necessary to understand the effect of live animal traits on meat tenderness. Beef tenderness has been identified as one of the most important buying considerations to consumers (Lusk et al., 2001, Savell et. al., 1987); however, there is much unexplained variation in beef tenderness within USDA quality grades (Smith et al., 1986). It has been well documented that chronic health problems such as bovine respiratory disease and SV liver abscesses can decrease beef yield and quality grades (Brown and Lawrence, 2010, Gardner et al., 1999). However, there is limited reported research evaluating the effects of liver abscesses or disease status on meat tenderness and sensory attributes. Gardner et al. (1999) reported no differences in WBSF values of LM steaks from animals with respiratory tract lesions at slaughter compared to animals without respiratory tract lesions when steaks had been aged for 14 or 21 d. However, a difference in tenderness at 7 d of aging was reported, with carcasses from animals with active respiratory tract lesions having higher WBSF values when compared to their normal counterparts, 4.0 and 3.6 kg respectively, but the authors indicated this difference was likely due to marbling differences within USDA quality grades between the groups. It is unknown why this tenderness difference was only observed at d 7 and not at later time points. Many studies have shown that steak tenderness increases as postmortem aging periods increase (Eilers et al., 1996, Lepper-Blilie et al., 2015, Martin et al., 1971). Currently, there is no standard amount of time that beef is aged postmortem before consumption, however the 2010 National Beef Tenderness Survey found that 21.6 d was the average post-fabrication aging period for strip loins at retail establishments before consumer purchase (Guelker et al., 2013).

Brown and Lawrence (2010) reported decreased 12th-rib fat depth for all liver abscess scores compared to healthy counterparts, along with decreased HCW yield grade, and marbling

scores associated with SV liver abscesses. The results of this study and those found by Gardner et al. (1999) and Brown and Lawrence (2010) imply that infections such as respiratory disease and liver abscesses negatively affect carcass characteristics such as HCW and marbling scores, but have no effect on meat tenderness or sensory attributes.

A linear increase of beef tenderness has been shown with increasing USDA quality grades (McBee and Wiles, 1967; Guelker et al., 2013). The 2010 National Beef Tenderness Survey found that overall, S steaks had greater WBSF values than C steaks, but data specifically for strip loin steaks was not reported (Guelker et al., 2013). While this research did not find an increase in tenderness, as measured by WBSF and SSF, not all previous research has found increased tenderness with increased quality grade (Romans et al., 1965; Miller et al., 1997). Warner-Bratzler Shear Force values not differing between S and C steaks is consistent with findings of Miller et al. (1997) where WBSF values were 2.39 and 2.29 respectively. This could be due to marbling being on a continuous scale, with an arbitrary boundary placed between S and C, resulting in marbling scores relatively similar to one another that are in different USDA quality grades. Current USDA quality grade standards place a high value on marbling scores to determine quality grade, but much inconsistency within quality grade for beef palatability is still seen (Miller et al. 1997).

Many studies have shown increased palatability traits with increased marbling scores (Tatum et al., 1982; Wheeler et al., 1994; Guelker et al., 2013) and is consistent with the results from the present study, where C steaks were more desirable than S steaks for initial and sustained juiciness, myofibrillar tenderness, overall connective tissue amount, and overall tenderness. Increased USDA quality grade is associated with increased palatability traits, which was also in agreement with the present study. In contrast, the 2010 National Beef Tenderness

Survey found no differences in palatability traits when comparing USDA S and C loin steaks (Guelker et al., 2013).

Warner-Bratzler Shear Force values and SSF values were relatively high for this study compared to other studies for these quality grades. The cause of this trend is unknown, but is most likely attributed to pre-slaughter effects such as genetic or other factors outside of the scope of this project. Warner-Bratzler Shear Force and SSF values were consistent with one another for steaks

Conclusion

These data suggest that liver abscesses do not impact meat tenderness, flavor, or other sensory attributes. This is beneficial knowledge for the livestock and meat industries given the current high prevalence of liver abscesses. In the future, it is possible that some liver abscess prevention methods, such as tylosin phosphate may be more heavily regulated. Other liver abscess preventions are currently being investigated, but none are widely used in the cattle feeding industry. While many of the ramifications of removing tylosin phosphate from finishing rations are unknown, it is now known that meat tenderness will not be impacted if prevalence of liver abscesses increases.

Although there were no differences in meat tenderness due to liver abscess score, liver abscesses still have a significant impact on margins in the beef industry due to decreased feedlot performance and marbling. Research on liver abscess prevention without the aid of antimicrobials is still warranted. Further possible extensions of this work include the effect of other animal health concerns on meat quality and length of postmortem aging on tenderness differences due to liver abscesses.

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Tables

Table 2.1 Least squares means and SEM for the effect of liver abscess score on Warner-Bratzler Shear Force, Slice Shear Force, and cook-loss for USDA Low Choice and Select beef strip loin steaks of finished feedlot steers not fed tylosin phosphate.

Item	Treatments									
	Quality Grade				Liver Abscess Score ¹				QG×LA	
	Select (n = 57)	Low Choice (n = 62)	SEM ¹	P-Value	None (n = 43)	Mild (n = 40)	Severe (n = 36)	SEM	P-Value	P-Value
WBSF ² , kg	4.53	4.22	0.13	0.09	4.42	4.34	4.35	0.17	0.91	0.38
SSF ³ , kg	28.48	26.93	0.39	0.39	29.02	26.69	27.41	1.62	0.52	0.61
Cook Loss, %	15.96	16.03	0.87	0.87	16.42	15.57	16.00	0.37	0.21	0.15

^{ab}Means within a row with different superscripts differ at the $P \leq 0.05$ significance level.

¹None- healthy liver, no abscess

Mild- 1 abscess less than 2 cm in diameter to 4 abscesses less than 4 cm in diameter

Severe- 1 abscess greater than 4 cm in diameter or greater than 4 small abscesses

²WBSF = Warner-Bratzler Shear Force

³SSF = Slice Shear Force

Table 2.2 Least squares means and SEM for the effects of liver abscess score on sensory analysis panel items for USDA Low choice and Select beef strip loin steaks in finished feedlot steers not fed tylosin phosphate.

Item	Treatments									
	Quality Grade				Liver Abscess Score ²				QG×LA	
	Select (n = 57)	Low Choice (n = 62)	SEM ¹	P-Value	None (n = 43)	Mild (n = 40)	Severe (n = 36)	SEM ¹	P-Value	P-Value
Initial Juiciness ³	55.11 ^a	59.06 ^b	1.42	0.01	57.83	56.50	56.93	1.61	0.71	0.37
Sustained Juiciness	46.20 ^a	49.58 ^b	1.26	0.01	48.18	47.62	47.86	1.45	0.94	0.66
Myofibrillar Tenderness	57.51 ^a	62.41 ^b	1.53	0.02	59.84	60.84	59.21	1.90	0.81	0.03
Connective Tissue Amount	17.05 ^a	13.89 ^b	1.38	0.03	15.24	14.84	16.33	1.60	0.70	0.06
Overall Tenderness	53.96 ^a	58.81 ^b	1.65	0.03	56.62	57.20	55.33	2.05	0.79	0.08
Beef Flavor Identity	47.05	48.39	0.87	0.27	48.39	47.67	47.10	1.10	0.68	0.10
Off Flavor	0.91	0.60	0.31	0.38	0.84	0.61	0.81	0.37	0.85	0.65

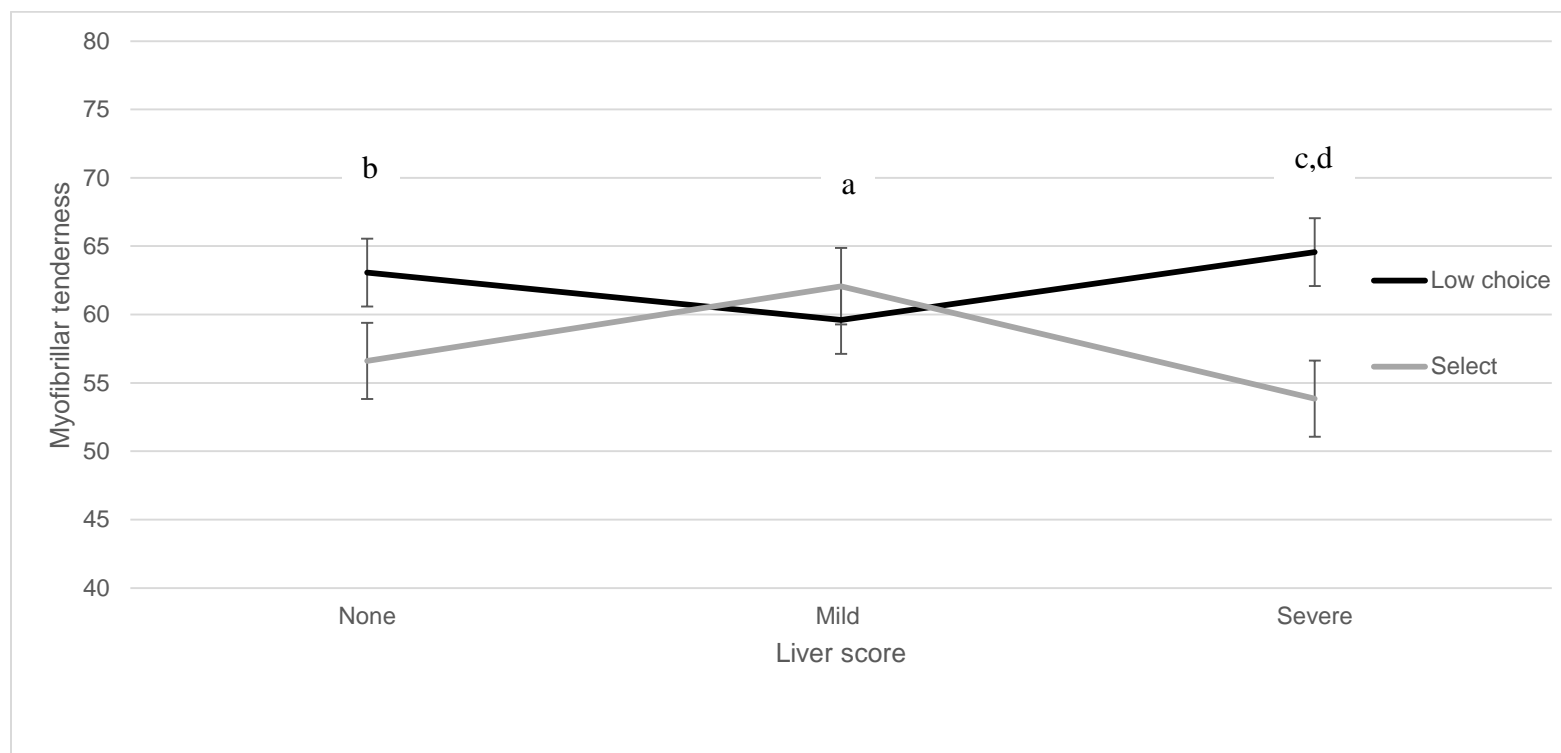
^{ab} Means within a row with different superscripts differ at $P \leq 0.05$ significance level.

¹None- healthy liver, no abscess; Mild- 1 abscess less than 2 cm in diameter to 4 abscesses less than 4 cm in diameter; Severe- 1 abscess greater than 4 cm in diameter or greater than 4 small abscesses

³Sensory Scores: 0 = Extremely dry/tough/non/bland; 100 = Extremely juicy/tender/abundant/intense; 50 = neither dry nor juicy, neither tough nor tender.

Figures

Figure 2.1 Least squares means of the effect of liver abscess score on myofibrillar tenderness by USDA quality grade for beef strip loin steaks from finished feedlot steers not fed tylosin phosphate on a continuous 1-100 scale.



a, b, c, d, e Means with common letters differ at significance level of $P \leq 0.05$.

Liver Score Descriptors: None = healthy liver, no abscesses ; Mild = 1 abscess less than 2 cm in diameter to 4 abscesses less than 4 cm in diameter; Severe = 1 abscess greater than 4 cm in diameter or greater than 4 small abscesses

Number of Observations: Low choice-none: n=22; Low choice-mild: n=20; Low choice-severe: n=20; Select-none: n=21; Select-mild: n=20; Select-severe: n=16

Chapter 3 - Subcutaneous Injection Safety Study for Cattle

Inoculated with VaxLiant[®] Adjuvants Assembled with Vaccine

Factions of *Fusobacterium necrophorum*.

Abstract

The primary objective of this study was to record any adverse reactions in cattle following vaccination and revaccination with 3 autogenous *Fusobacterium necrophorum* vaccine formulations with different adjuvants compared to cattle that receive a placebo control. Bos Taurus steers (n=40; approx. 340 kg) were used to test the safety of 3 autogenous *F. necrophorum* vaccines. Each vaccine contained 6 strains *Fusobacterium necrophorum* sp. *necrophorum* bacterim in combination with an adjuvant. Vaccine A included adjuvant 1 at a 20% inclusion rate, vaccine B included adjuvant 2 at a 10% inclusion rate, vaccine C included adjuvant 3 at a 15% inclusion rate, and vaccine d contained the antigen and a sterile buffered saline. Each vaccine was administered subcutaneously at a 2 cc dose. Cattle were vaccinated on day 0 and revaccinated on day 14. Cattle were checked for injection site nodules and rectal temperatures were taken on days 1, 2, 14, 15 and 16 of the study. If a nodule was present, calipers were used to measure nodule diameter which was also recorded. The PROC GLIMMIX procedure of SAS was used to analyze data. All cattle were healthy for the duration of the study. There were no differences in rectal temperature for cattle vaccinated with any of the vaccine formulas on day 0, 1, or 2 ($P > 0.05$). There were no differences in rectal temperatures between cattle administered any of the vaccine formulations on day 14, 15, or 16 ($P > 0.05$). Between day 14 and 15, cattle receiving vaccine A had a greater increase in body temperature than cattle that

received vaccine D. Cattle that received vaccine D had fewer observed injection site nodules than vaccines A, B, or C. All 3 autogenous vaccine preparations proved to be safe with no adverse reactions seen other than small injection site nodules compared to the non-adjuvanted placebo vaccine. There were minimal changes or differences in rectal temperature of cattle due to vaccine formulation.

Introduction

Vaccination to prevent liver abscesses has been a widely researched area in animal health as an alternative to antimicrobial use. Two commercial vaccines have been on the market for liver abscess prevention including Fusogard and Centurion. These vaccines have shown to be effective in groups of cattle with low prevalences of liver abscesses, but are not effective with high prevalence groups (Jones et. al, 2004; Checkley et al., 2005; Fox and Thomson, 2009). As the US beef industry strives to reduce antibiotic use, and in turn, decrease the contribution to antimicrobial resistance issues, a non-antibiotic liver abscess prevention technique is needed.

AgriLabs (now Huvapharma) is the parent company of VaxLiant®, who has developed a group of adjuvants for use in veterinary biologics production and research. These adjuvants have been shown to be effective in combination with other antigens at increasing immunity to pathogens according to research done by AgriLabs. The USDA APHIS CVB approved the first of these adjuvants to be used in cattle and swine vaccines. The adjuvant formulas to be tested in the present study for cattle were a combination of adjuvants with *Fusobacterium necrophorum* sp. *necrophorum* for inoculation by subcutaneous administration. These adjuvants have also been approved for 21-day slaughter withdrawal in cattle via the subcutaneous and intramuscular route.

The primary objective of this study was to record any adverse reactions in cattle following vaccination and revaccination with 3 autogenous *F. necrophorum* vaccine

formulations with different adjuvants compared to cattle that receive a placebo control. A secondary objective was to observe cattle for changes in rectal temperature or formation of injection site lesions due to the different vaccine formulations.

Materials and methods

The Kansas State University Institutional Animal Care and Use Committee approval was obtained via protocol number 3976 for this study.

Cattle:

Bos Taurus steers (n=40; approximate average weight of 340 kg) were used to test the safety of 3 autogenous *Fusobacterium necrophorum* vaccines. This study was conducted in the summer of 2018. Cattle were housed as a group and acclimated to the pen for 21 days before study initiation. The pen was a dirt floor lot with a concrete apron and automatic waterers located at a private facility in Pottawatomie County Kansas. Cattle were fed once daily and had ad libitum access to feed. Cattle were randomly assigned to one of four vaccination test groups: A) *Fusobacterium necrophorum* antigen (Fn Ag) with adjuvant 1 at 20%; B) Fn Ag with adjuvant 1 at 10%; C) Fn Ag with adjuvant 2 at 15%; or D) Fn Ag in phosphate buffered saline (no adjuvant). Ten cattle were allotted to each vaccine treatment group. Cattle were vaccinated on day 0 and revaccinated on day 14 during the 16 day trial with one vaccine. Rectal temperatures were taken on day 0, 1, 2, 14, 15 and 16 using a GLA Model M900H rectal probe thermometer (GLA Agricultural Electronics, San Luis Obispo, California). Cattle were monitored daily for any adverse reactions, clinical signs of illness or injury.

Vaccination:

On day 0, a 3 × 3 in² area of hair was clipped using a size 10 clipper blade on the left side of the neck for the initial vaccination. New disposable 3 mL syringes with 16 gauge, 5/8 in.

needles were used for injections on each animal. A 2 cc dose of vaccine treatment assigned to each animal was administered subcutaneously. On day 14, the cattle's hair was clipped similarly on the right side of the neck and cattle were revaccinated using the same procedures as those used for the initial vaccination. Cattle necks were checked for injection site nodules on day 1, 2, 14, 15 and 16 of the study and presence was recorded. If a nodule was present, calipers were used to measure nodule diameter which was also recorded.

Statistics:

The PROC GLIMMIX procedure of SAS (version 9.4; SAS Inst. Inc., Carry, NC) was used to analyze data. Comparisons between least square means for treatments utilized the PDIF option of the LSMEANS statement. Nodule palpation size and rectal temperature were measured with animal as the experimental unit. Treatment was included in the model as a fixed class effect. Statistical significance was determined at $P \leq 0.05$.

Results and discussion

Adverse reactions:

All cattle were healthy for the duration of the study. No animals had to be removed from the study for any reason. These results indicate that this vaccine does not adversely impact animal health during the time immediate following vaccination and revaccination.

Rectal Temperature:

There were no differences in rectal temperature for cattle vaccinated with any of the vaccine formulas on day 0, 1, or 2 body temperature ($P > 0.05$; Figure 3.1). There were also no differences between cattle due to vaccination group for the change in rectal temperature from baseline (day 0) to day 1 or 2 ($P > 0.05$; Table 3.4). Mean rectal temperature for all cattle

regardless of vaccination group increased numerically between day 0 and 1, and decreased between day 1 and 2.

The rectal temperatures of the test cattle taken day 0, 1 and 2 were abnormally elevated over expected rectal temperatures of healthy cattle. High ambient temperatures and temperature humidity index (Figure 3.3) on the days prior to and through this recording period attributed to the elevated rectal temperatures in the test cattle. Nighttime temperatures in the days leading up to the initiation of the trial were elevated and cattle were unable to offload heat. Maximum ambient temperatures for the day before trial initiation was 97° F with 76% humidity and remained high for both temperature and humidity for d 1 and 2 of the study. This resulted in elevated body temperatures in the morning, being exacerbated by the increasing temperatures throughout the day. Temperature humidity index has been positively correlated to rectal temperature, with rectal temperature being indicative of core body temperature (Alhidary, et al., 2012; Bernardini, et al., 2012; Kaufman, et. al., 2018).

There were no differences in rectal temperatures between cattle administered any of the vaccine formulations on day 14, 15, or 16 ($P > 0.05$; Figure 3.2). Between day 14 and 15, cattle receiving vaccine A had a greater increase in body temperature than cattle that received vaccine D (1.17 vs -0.24 °F; $P < 0.05$). There were no differences in rectal temperature among cattle receiving any of the vaccine formulations for differences in rectal temperature differences between days 15 and 16 or days 14 and 16.

Rectal temperatures of test cattle were closer to expected normal range of healthy during revaccination and the following observations. The ambient temperatures and THI were lower during this time period.

Injection Site Observations:

Table 3.2 contains categorical injection site observation data. Cattle that received vaccine D had fewer observed injection site nodules than vaccines A, B, or C. Cattle receiving vaccine A or B had larger injection site nodules on day 1 than cattle receiving vaccine formulation D ($P < 0.05$; Table 3.3). There were no differences in diameter of injection site nodules for cattle receiving vaccine A, B or C on day 2, 15 or 16 of the study. Cattle vaccinated with the placebo (no adjuvant) formulation had smaller diameter injection site nodules on day 1, 2, 15 and 16 of the study. Anecdotally, many injectable products used in the beef industry, both vaccines and antibiotics will temporarily leave injection site nodules with any complications arising from them.

Two steers still had a palpable nodule on day 14 following the initial vaccination (one steer receiving vaccine A (1.0 cm nodule) and a steer receiving vaccine C (2.3 cm nodule)).

Feed Intake Observations:

Since all cattle were housed together, feed intake between treatments cannot be compared. Due to cattle temperament, it was difficult to monitor group appetite following vaccination. Daily total feed offerings to the pen are in Figure 4.4. Cattle were fed a grower diet consisting of silage and corn gluten feed. The day of the initial vaccination, the cattle did not clean the bunk, so day 1 of the study, cattle were cut back from 681.8 to 454.5 kg. Day 2, cattle did not clean the bunk, so they were fed 454.5 kg again. For the remainder of the study, cattle cleaned the bunk daily including the day of revaccination and days 15 and 16. The initial drop in feed intake was most likely due to THI and processing stress combined and not related to the vaccines themselves. During the revaccination time, cattle cleaned the bunk daily. Cattle were fed step-up increments consistent with the grow yard protocols throughout the study duration.

Conclusions

All 3 autogenous vaccine preparations proved to be safe with no adverse reactions seen other than small injection site nodules compared to the non-adjuvanted placebo vaccine. No animals had to be removed from the trial for any adverse reactions. There were minimal changes or differences in rectal temperature of cattle due to vaccine formulation. Vaccines with the adjuvant included in the formulation had increased presence of injection site nodules the next day or two post-vaccination compared to cattle vaccinated with the non-adjuvant control vaccine. These vaccine injection site nodules resolved to nearly undetectable levels by two weeks after the initial vaccine. Cattle vaccinated with vaccine A or B had larger diameter nodules on their neck on day 1 post-vaccination compared to cattle receiving vaccine D, the placebo. However on day 2 and later, cattle vaccinated with vaccine A, B or C had larger diameter nodules on the neck than cattle that received vaccine D.

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Tables

Table 3.1 VaxLiant® Adjuvants combinations assembled with vaccine factions of *Fusobacterium necrophorum* composing the four treatment groups.

TREATMENT GROUP	PRODUCT ¹	NUMBER OF CATTLE
A	Fn Ag (IVP)/Adjuvant 1 20%	10
B	Fn Ag (IVP)/Adjuvant 1 10%	10
C	Fn Ag (IVP)/Adjuvant 2 15%	10
D	FN AG (MPC) PB SALINE	10

¹All product was administered subcutaneously.

Table 3.2 Number of injection site reactions following initial vaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

	VACCINATION GROUP							
	Day 1				Day 2			
	A (n=10)	B (n=10)	C (n=10)	D (n=10)	A (n=10)	B (n=10)	C (n=10)	D (n=10)
NODULE	7	7	5	2	9	9	7	1
MILD EDEMA	0	2	2	1	1	1	3	0
NO NODULE	3	1	3	7	0	0	0	9

Table 3.3 Number of injection site reactions following revaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

	REVACCINATION GROUP							
	Day 15				Day 16			
	A (n=10)	B (n=10)	C (n=10)	D (n=10)	A (n=10)	B (n=10)	C (n=10)	D (n=10)
NODULE	10	8	9	3	10	9	10	2
MILD EDEMA	0	2	1	2	0	1	0	1
NO NODULE	0	0	0	5	0	0	0	7

Table 3.4 Least squared means for injection site nodule size by vaccine treatment group following vaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

		VACCINATION GROUP				SEM ¹	P-Value
NODULE SIZE (CM)		A	B	C	D		
	D1	1.89 ^a	2.00 ^a	1.25 ^{ab}	0.34 ^b	0.392	0.02
	D2	2.50 ^a	2.94 ^a	2.05 ^a	0.17 ^b	0.341	<0.01
	D15	3.21 ^a	2.20 ^a	2.55 ^a	0.41 ^b	0.295	<0.01
	D16	3.79 ^a	3.26 ^a	3.56 ^a	0.36 ^b	0.322	<0.01

^{ab}Means within a row with different superscripts differ at $P \leq 0.05$ significance level.

¹Standard Error of the Mean

Table 3.5 Least squared means for temperature differences (°F) between vaccine administration and 24 to 48 hours after vaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

DIFFERENCE IN STUDY DAYS ²		VACCINATION GROUP				SEM ¹	P -Value
		A	B	C	D		
	0-1	0.30	0.40	0.26	0.71	0.354	0.80
	0-2	-0.94	-1.09	-1.80	-0.88	0.336	0.23
	1-2	-1.24	-1.49	-2.06	-1.59	0.338	0.33
	14-15	1.17 ^a	0.78 ^{ab}	0.23 ^{ab}	-0.24 ^b	0.315	0.02
	14-16	0.56	0.65	0.29	-0.08	0.291	0.30
	15-16	-0.61	-0.13	0.06	0.16	0.364	0.46

^{ab} Means within a row with different superscripts differ at $P \leq 0.05$ significance level.

¹Standard Error of the Mean

²This is the initial temperature subtracted from the later day's temperature (ex: 0-1 is average temperature for day 0 subtracted from average temperature from day 1).

Figures

Figure 3.1 Rectal temperature at vaccination, and day 1 and 2 following vaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

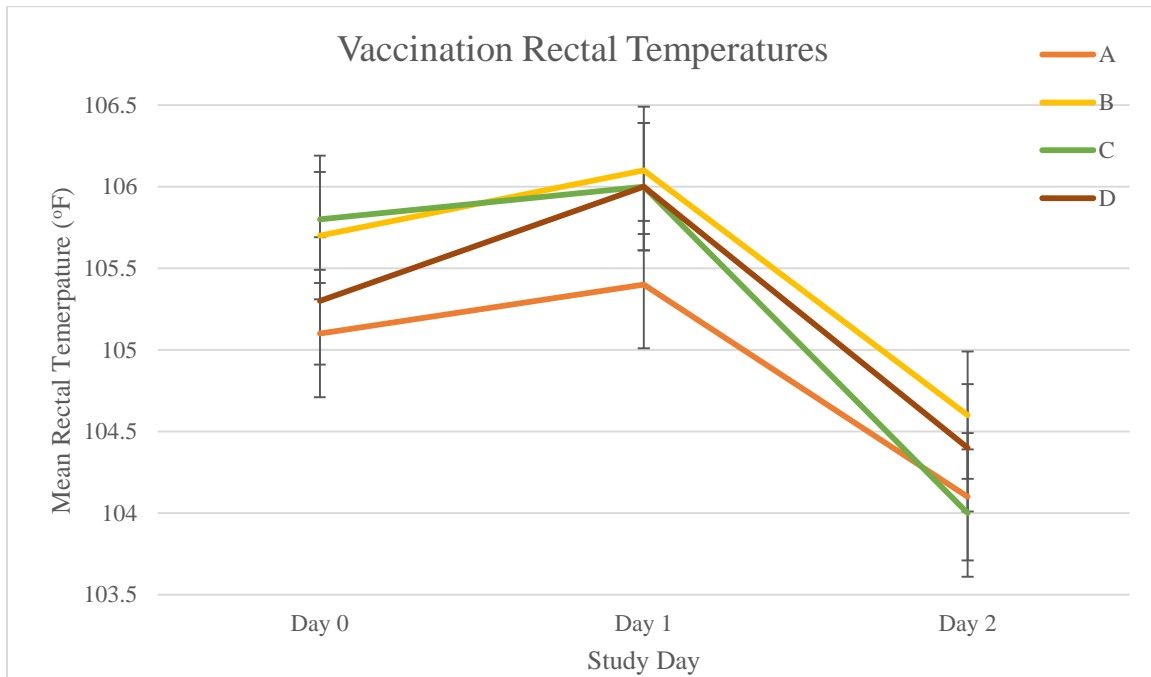


Figure 3.2 Rectal temperature at revaccination, and day 1 and 2 following revaccination with vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.

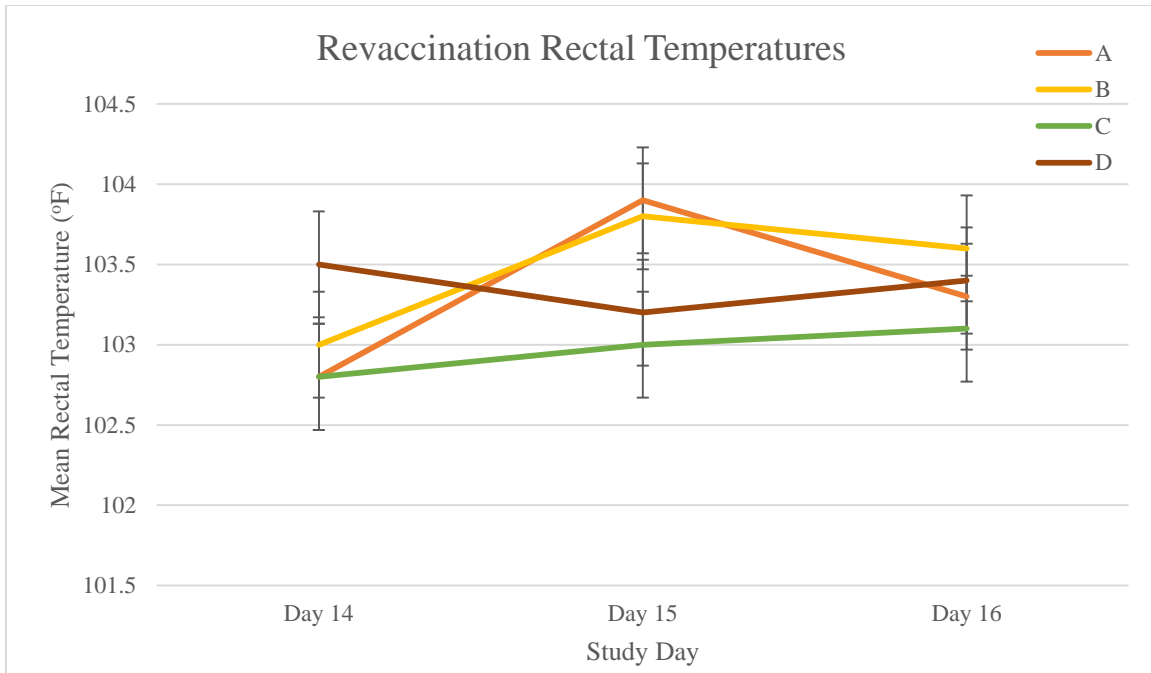
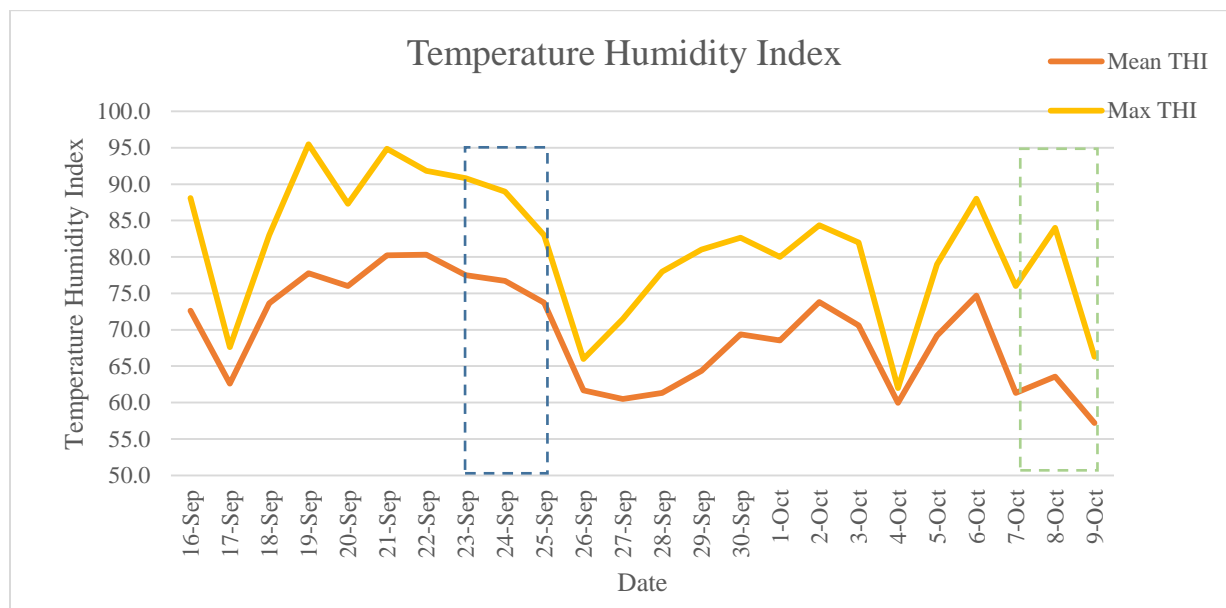
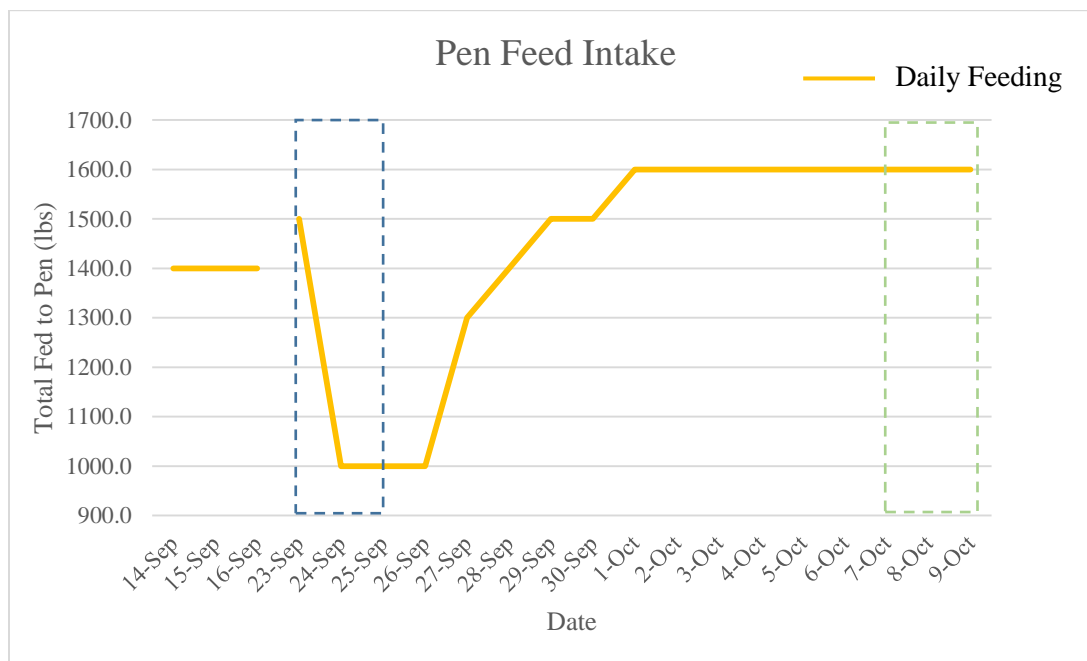


Figure 3.3 Maximum and average Temperature Humidity Index (THI) prior to and throughout the duration of the study investigating vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.



^aVaccination data collection period has a blue box surrounding it and revaccination data collection periods has a green dotted box.

Figure 3.4 Feed offered to the pen daily throughout the duration of the study investigating vaccines containing VaxLiant® Adjuvants assembled with factions of *Fusobacterium necrophorum*.



^aVaccination data collection period is in the blue dotted box and revaccination data collection period is in the green dotted box.

^bData was not collected between Sept. 16th and 23rd. Through Sept. 16th cattle were cleaning up 1400 lbs daily, and they were bumped up to 1500 lbs daily by Sept. 23rd.

Figure 3.5 Injection site nodule (3.0 cm) on the day after vaccination (d 1) in steer administered vaccine B containing a VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum*.



Figure 3.6 Injection site nodule (2.5 cm) on the day after vaccination (d 1) in steer administered vaccine A containing VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum*.



Figure 3.7 Injection site nodule (3.1 cm) the day after revaccination (d 15) in steer administered vaccine C, containing VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum*.

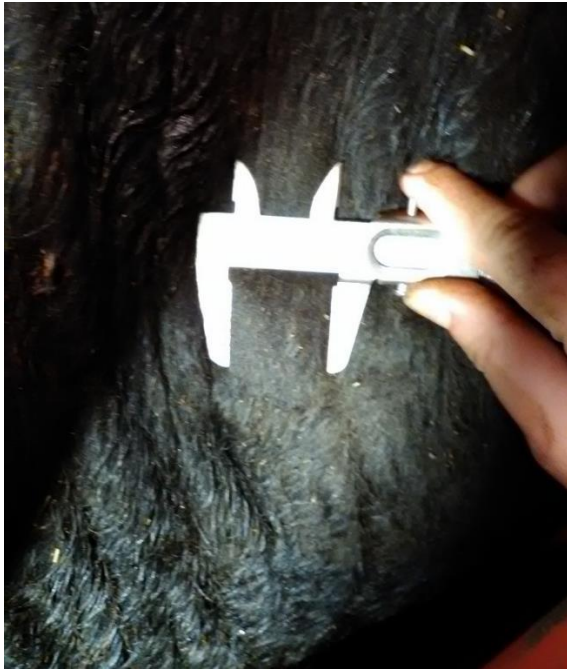


Figure 3.8 Injection site nodule (5.0) two days after revaccination (d 16) in steer administered vaccine A, containing VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum*.



Figure 3.9 Injection site nodule (4.6 cm) two days following revaccination (d 16) in steer administered vaccine C, containing VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum*.



Figure 3.10 Same steer as Image 3.9 two days after revaccination (d 16) VaxLiant® Adjuvant assembled with factions of *Fusobacterium necrophorum* without calipers



Chapter 4 - The effects of an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine on health, production and carcass characteristics including liver abscess incidence in feeder cattle

Abstract

Vaccination against the causative agent of liver abscesses, *Fusobacterium necrophorum* (*F. necrophorum*) shows potential to reduce liver abscesses in feeder cattle. This study was a production trial investigating the efficacy of the autogenous vaccine against *F. necrophorum* at reducing liver abscesses in feeder cattle, and the effects of the vaccine on animal health, gain, and carcass data. Natural-fed cattle (n=996 hd; 848 ± 72 lbs) were enrolled upon feedlot processing and randomly assigned to treatment groups of vaccinates or controls. Vaccinates were administered a 2cc dose of the autogenous *F. necrophorum* vaccine and were revaccinated 14-28 days later. Control and vaccinated cattle were commingled within pens in a 1:1 fashion and no further interventions were implemented during the finishing period. All cattle were fed as part of a natural program, therefore Tylosin was not included in the diet. At slaughter, carcass data and liver abscess scores were collected using the Elanco Liver Check scoring system. Liver abscess prevalence did not differ between controls and vaccinates (37.4 vs 34.1%; $P = 0.10$), nor did prevalence of severe liver abscesses (15.4 vs 13.7%; $P = 0.15$). There were no differences between control and vaccinated cattle for yield grade, quality grade, enrollment weights, HCW, estimated slaughter weight, average daily gain or days on feed ($P > 0.14$). Results indicate this vaccine does not reduce liver abscess prevalence in feedlot cattle and does not have an impact on carcass parameters.

Introduction

Liver abscesses are a common issue in the United States cattle feeding industry, with the most recent National Beef Quality Audit recording 17.8% of the fed cattle observed at abattoirs to have had liver abscesses (Eastwood, et al., 2017). The most common bacterial pathogen isolated in liver abscesses in cattle is *Fusobacterium necrophorum*, sp. *necrophorum* (*F. necrophorum*), which has been isolated from up to 100% of liver abscesses seen at the time of slaughter (Nagaraja and Chengappa, 1998; Nagaraja et al., 1999). Vaccination against this causative agent has previously shown potential to reduce liver abscesses in feeder cattle. Previous vaccines have been found to be effective in groups of cattle with relatively low prevalence of liver abscesses (Checkley, 2005; Jones, 2004). This study investigated a vaccine against *F. necrophorum* sp. *necrophorum* that contained an adjuvant from Huvepharma (previously AgriLabs VaxLiant®) that was developed for use in veterinary biologics. Previous company research has demonstrated favorable results in improved immune response and a superior safety profile utilizing various antigens in combination with this adjuvant. This product has documented adjuvant licensure safety and a 21 day slaughter withdrawal. This vaccine was previously proven safe for use in cattle, not resulting in any observed adverse effects in a 40 head safety study.

This study was a large scale production trial investigating 1) the efficacy of the autogenous vaccine against *F. necrophorum* at reducing liver abscesses in feeder cattle, and 2) the effects of the autogenous vaccine against *F. necrophorum* on animal health, feedlot and carcass data. Feeder cattle were enrolled in the study between April and June of 2018 at 2 cooperating feedyards near Scott City, Kansas. A total of 3,591 hd were enrolled in the study, 947 hd split between 7 lots of cattle at Feedyard A and 2,644 hd split between 23 lots at

Feedyard B. Cattle were harvested between September of 2018 and March of 2019 at commercial abattoirs in Southwest Kansas and Nebraska.

Materials and methods

The Kansas State University Institutional Animal Care and Use Committee approval was obtained via protocol number 4085 for this study.

Enrollment:

Lots of natural cattle were selected at feedlot arrival to participate in the study. Within pen, cattle were randomly allotted to treatment, so that each pen was comprised of half vaccinated cattle and half control cattle. Treatments consisted of control cattle that did not receive the *F. necrophorum* autogenous vaccine and vaccinated cattle that received a 2cc injection subcutaneously at the time of processing upon arrival, and a revaccination 14-28 days after the initial vaccination. Vaccines were transported, stored and administered according to manufacturer instructions. All cattle received the processing vaccines typical for the feedyard in addition to the study treatment allocated to them and all cattle, including controls were brought through the working facility upon vaccination and revaccination. The randomization procedures and vaccinations were observed by trained personnel under the supervision of the feedlot's veterinary consultant. After processing, cattle were put into their home pen for routine health and behavior observations and no further interventions were implemented other than what was routine for the feedlot. Cattle were fed as part of a natural program, therefore diets did not contain tylosin. Finishing ration formulations can be found in Table 5 for Feedyard A and Table 6 for Feedyard B. All cattle received an electronic individual animal identification (EID) and the treatment they received in the processing barn was recorded with the EID for future tracking of the cattle health and performance using the animal health computer system at the feedlot.

Animal health data was gathered retrospectively from the animal health computer after lot closeout.

Carcass Data:

At slaughter, a carcass data collection team from the West Texas A&M University Beef Carcass Research Center collected individual carcass data including liver abscess scores visually, and hot carcass weight (HCW), yield grade and quality grade from the abattoirs' grading system. Livers were scored according to the Elanco Liver Check System with the following categories: 0 = free of abscesses, parasites, or other pathological abnormalities; A- = ≤ 2 abscess ≤ 2 cm in diameter or resolved abscess scars; A = 2 to 4 abscesses 2 to 4 cm in diameter; A+ = ≥ 1 abscesses > 4 cm in diameter or > 4 abscesses > 2 cm in diameter; and A+Adhesions = Abscesses adhered to the diaphragm, other organs, or the abdominal cavity. If livers were condemned for any reason, no liver score was obtained. Estimated slaughter weight was calculated using hot carcass weight and an average dressing percentage of 63%.

Due to a communication failure, 5 lots of (Feedyard B) cattle were shipped without notifying researchers, so liver abscess data was not collected on these 738 cattle. Yield grade, quality grade and HCW was obtained from the packer for 4 of these lots.

Data Analysis:

This study implemented a randomized complete block design with feedlot and pen being the blocking factors. Individual animal was the experimental unit. Study endpoints were analyzed using either the generalized linear mixed model or the linear mixed model. Fixed effects of the model included feedlot (A, B) and treatment (VAC, CON). Bodyweight at enrollment served as the covariate. Pen nested within feedlot was the random effect of the model. Binary endpoints, including incidence for liver abscess (Normal, A- vs. A, A+, A+Adh), severe

liver abscess (Normal, A-, A vs. A+ A+Adh), morbidity and mortality, were analyzed under the generalized linear mixed model with logit link. Ordinal endpoints, including liver abscess scores, USDA quality grade and yield grade, were analyzed under the generalized linear mixed model with cumulative logit link. HCW and ADG are continuous endpoints. These two endpoints were analyzed under the linear mixed model with identity link. For binary and ordinal endpoints, rates of incidences and the standard errors were reported for each treatment. Treatment effect was evaluated via the 2-sided test for non-one (cumulative) odds ratio. For continuous endpoints, least square (LS) means and their standard errors were reported for each treatment. Treatment effect was evaluated via the 2-sided test for non-zero mean difference. Statistical analysis was performed using the SAS (Version 9.4; SAS Inst. Inc., Cary, NC) PROC GLIMMIX.

Results

Liver abscess data:

Liver abscess data were obtained and analyzed for 2,344 hd and can be found in table 4.2 and figure 4.1. Table 4.2 summarizes liver data by lot in counts and prevalence. Figure 4.1 displays the prevalence of liver abscess categories by treatment group. Overall prevalence of liver abscesses by lot ranged from 4.4 to 80.3%. Liver abscess prevalence did not differ between controls and vaccinates (37.4 vs 34.1%; $P = 0.10$), nor did prevalence of severe liver abscesses (15.4 vs 13.7%; $P = 0.15$).

Carcass Data:

Carcass data was obtained for 2,992 head and can be found in Figures 4.2 and 4.3. Yield grade did not differ between control and vaccinated cattle ($P = 0.35$) nor did the quality grade ($P = 0.46$). HCW did not differ between control and vaccinated cattle (777.4 vs 774.7 lb; $P = 0.14$).

Performance and Health Data:

Table 4.3 summarizes performance and health data by treatment. There were no differences between control and vaccinated cattle for enrollment weights (749 vs 746 lb; $P = 0.52$), nor for HCW (777.4 vs 774.7 lb; $P = 0.14$). Average daily gain did not differ between control and vaccinated cattle (2.97 vs 2.94 lb; $P = 0.15$). There was no difference between controls and vaccinates for morbidity (7.3 vs 8.5%; $P = 0.20$) or mortality (2.1 vs 1.5%; $P = 0.23$).

Discussion

This research agrees with Fox and Thomson (2009) who tested 2 other vaccines (Fusogard and Centurion) against liver abscesses in beef cattle caused by *F. necrophorum*. They found that neither of these vaccines reduced liver abscess prevalence or severity in a group of cattle that had high liver abscesses prevalence (56% total abscess prevalence, 39% severe). Additionally in agreement with this research, Fox and Thomson found that these neither Fusogard nor Centurion affected hot carcass weight, yield grade, or quality grade.

Previously, vaccines have been found to decrease prevalence and severity of liver abscesses in groups of cattle with low prevalence of liver abscesses at the time of slaughter (Checkley, et al., 2005; Jones et al., 2004). A *T. pyogenes* - *F. necrophorum* bivalent bacterin-toxoid vaccine, Centurion (Originally Schering-Plough Corp, now Merck Animal Health; Omaha, NE) was found in two studies to reduce liver abscess prevalence by 37.5 to 48.4% (16% vaccines vs 31% controls in study one and 30% vaccinates vs 48% controls in study two) in cattle on a high grain ration. The vaccine contained an inactivated *F. necrophorum* leukotoxin and *T. pyogenes* pyolysin that was used in combination with a water-in-oil adjuvant, however this product is no longer available commercially.

Fusogard (Elanco Animal Health US, Inc, Greenfield, IN) is a currently available *F. necrophorum* bacterin vaccine. This vaccine has been shown to be effective in groups of cattle with low overall prevalence of liver abscesses (decreasing overall abscesses from 10% to 2%), but is less effective in groups of cattle with an increased overall prevalence (decreasing abscesses from 30% to 24%; Checkley, et al., 2005) or not effective at all as found by Fox and Thomson (2009) in cattle with a 59% abscess prevalence.

The vaccine researched in this article is the first autogenous liver abscess prevention vaccine to be investigated. Historically for other conditions, autogenous vaccines have little efficacy data available. O’Conner et al. (2011) found that an autogenous vaccine against *Moraxella bovis* was not effective at controlling naturally-occurring Infectious Bovine Keratoconjunctivitis (pinkeye) in calves. While these disease processes are very different, this demonstrates that although little research is published on the efficacy of autogenous vaccines, the research that is available widely does not support their use.

The vaccine being researched here is an autogenous bacterin vaccine, meaning that the vaccine contained killed bacteria collected from the geographical area where the vaccine was used. The bacteria was collected at the time of slaughter from lots of cattle that had mild to severe liver abscesses. It is possible that the strains of *F. necrophorum* used in this vaccine did not match those strains affecting the majority of cattle on trial. Little is known about strain variation of *F. necrophorum* regionally and exactly how this affects liver abscess prevalence and severity of feedlot cattle but it is speculated that the soil microbiome differences contributes to the regional variation in liver abscess prevalence (Weinroth, et al., 2019). Cattle likely arrive at the feedlot with *F. necrophorum* strains in their rumen from their ranch or backgrounding operation of origin, which may differ from the strains collected and used in the vaccine.

Many vaccines contain components that target specific virulence factors of bacteria as opposed to a bacterin vaccine like this one that contains entire killed bacteria cells. With some bacteria this can lead to an increased efficacy at encouraging the body to mount an adequate immune response against virulent bacteria and may help explain the low efficacy of the vaccine in question. Although this vaccine was previously proven safe and no adverse reactions were seen in this large scale study, it is notable that component vaccines are also known for creating fewer adverse reactions than killed bacterin vaccines (Richeson et al, 2019). Endotoxins are in the cell wall of gram-negative bacteria and could be responsible for causing an anaphylactic shock reaction in a killed bacterin vaccine (Richeson et al., 2019).

To summarize these findings, currently there are no vaccines on the market that are effective at reducing liver abscess prevalence or severity in groups of cattle with high prevalence, including the autogenous vaccine researched in this article. More research is needed to find a vaccine that is capable of preventing liver abscesses. Targeting specific virulence factor genes appears to be a more effective approach at liver abscess prevention than killed bacterin vaccines.

Conclusions

Results from this experiment indicate that use of this autogenous vaccine against *F. necrophorum* did not impact liver abscess prevalence or severity, animal health, feedyard or carcass performance in cattle that had an average feedlot entry weight of 758 lbs. Further research is needed to find a vaccine that is capable of preventing liver abscesses in groups of cattle with high prevalence of liver abscesses.

Acknowledgement

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Tables

Table 4.1 Descriptive data of all lots for which data was obtained when investigating an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.

Feedlot	Lot	Head in Pen at Slaughter	Average In Weight (lbs)	Average HCW (lbs)	Sex
A	N455	84	844 ± 78	779 ± 72	Steers
A	N457	52	916 ± 76	770 ± 55	Heifers
A	N458	123	832 ± 87	739 ± 74	Steers
A	N459	131	824 ± 77	743 ± 70	Steers
A	N460	165	839 ± 56	797 ± 63	Steers
A	N461	174	828 ± 73	776 ± 74	Heifers
A	N462	166	841 ± 65	783 ± 66	Steers
B	7890	188	752 ± 64	814 ± 61	Heifers
B	7893	58	650 ± 52	834 ± 71	Heifers
B	7896	113	758 ± 69	769 ± 68	Heifers
B	7901	248	750 ± 66	841 ± 65	Heifers
B	7902	67	702 ± 42	827 ± 64	Heifers
B	7903	59	723 ± 55	859 ± 67	Heifers
B	7906	56	777 ± 85	852 ± 85	Mixed
B	7915	55	764 ± 65	846 ± 63	Heifers
B	7920	101	860 ± 65	836 ± 64	Steers
B	7921	206	782 ± 68	851 ± 70	Heifers
B	7933	67	713 ± 68	790 ± 75	Mixed
B	7934	30	847 ± 57	884 ± 70	Steers
B	7936	58	811 ± 101	900 ± 72	Steers
B	7941	38	474 ± 74	764 ± 67	Heifers
B	7942	37	516 ± 70	806 ± 66	Steers
B	7943	212	902 ± 67	903 ± 86	Heifers
B	7944	81	514 ± 50	786 ± 91	Mixed
B	7950	178	515 ± 50	728 ± 68	Mixed
B	7951	96	429 ± 51	723 ± 67	Mixed
B	7952	89	669 ± 86	779 ± 78	Heifers
B	7953	175	719 ± 71	797 ± 72	Heifers

Table 4.2 Overall counts and prevalence of liver abscesses by lot for the efficacy study of an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.

Lot		Counts							Prevalence, %					
		.	0	1	2	3	4	All	.	0	1	2	3	4
458	CON	1	38	7	3	5	9	63	1.6	60.3	11.1	4.8	7.9	14.3
	VAC	1	41	3	7	2	7	61	1.6	67.2	4.9	11.5	3.3	11.5
N455	CON	5	28	5	.	1	2	41	12.2	68.3	12.2	.	2.4	4.9
	VAC	.	38	3	.	2	2	45	.	84.4	6.7	.	4.4	4.4
N457	CON	2	19	3	1	.	1	26	7.7	73.1	11.5	3.8	.	3.8
	VAC	.	23	.	1	2	.	26	.	88.5	.	3.8	7.7	.
N459	CON	3	41	7	6	6	2	65	4.6	63.1	10.8	9.2	9.2	3.1
	VAC	.	46	7	3	6	4	66	.	69.7	10.6	4.5	9.1	6.1
N460	CON	9	13	29	14	13	5	83	10.8	15.7	34.9	16.9	15.7	6.0
	VAC	6	14	32	22	10	5	89	6.7	15.7	36.0	24.7	11.2	5.6
N461	CON	14	18	32	21	10	3	98	14.3	18.4	32.7	21.4	10.2	3.1
	VAC	13	17	26	14	17	7	94	13.8	18.1	27.7	14.9	18.1	7.4
N462	CON	14	50	5	3	3	9	84	16.7	59.5	6.0	3.6	3.6	10.7
	VAC	8	60	8	4	3	3	86	9.3	69.8	9.3	4.7	3.5	3.5
7893	CON	10	17	1	1	5	2	36	27.8	47.2	2.8	2.8	13.9	5.6
	VAC	6	19	2	3	2	3	35	17.1	54.3	5.7	8.6	5.7	8.6
7901	CON	32	101	7	3	5	1	149	21.5	67.8	4.7	2.0	3.4	0.7
	VAC	21	106	1	4	6	3	141	14.9	75.2	0.7	2.8	4.3	2.1
7902	CON	2	24	3	2	3	.	34	5.9	70.6	8.8	5.9	8.8	.
	VAC	2	25	3	2	2	.	34	5.9	73.5	8.8	5.9	5.9	.
7903	CON	14	18	2	1	.	.	35	40.0	51.4	5.7	2.9	.	.
	VAC	11	23	34	32.4	67.6
7915	CON	6	18	1	2	2	2	31	19.4	58.1	3.2	6.5	6.5	6.5
	VAC	1	17	2	6	3	2	31	3.2	54.8	6.5	19.4	9.7	6.5

7920	CON	10	24	6	8	7	1	56	17.9	42.9	10.7	14.3	12.5	1.8
	VAC	9	23	8	4	10	1	55	16.4	41.8	14.5	7.3	18.2	1.8
7933	CON	3	25	2	2	2	.	34	8.8	73.5	5.9	5.9	5.9	.
	VAC	7	22	2	.	3	1	35	20.0	62.9	5.7	.	8.6	2.9
7934	CON	19	11	.	.	.	1	31	61.3	35.5	.	.	.	3.2
	VAC	17	10	1	1	1	1	31	54.8	32.3	3.2	3.2	3.2	3.2
7936	CON	2	20	4	1	1	2	30	6.7	66.7	13.3	3.3	3.3	6.7
	VAC	7	12	6	1	1	3	30	23.3	40.0	20.0	3.3	3.3	10.0
7941	CON	7	12	2	1	2	2	26	26.9	46.2	7.7	3.8	7.7	7.7
	VAC	8	12	1	1	2	1	25	32.0	48.0	4.0	4.0	8.0	4.0
7942	CON	7	14	.	2	1	2	26	26.9	53.8	.	7.7	3.8	7.7
	VAC	5	15	.	.	2	.	22	22.7	68.2	.	.	9.1	.
7943	CON	8	40	19	12	33	2	114	7.0	35.1	16.7	10.5	28.9	1.8
	VAC	30	39	8	9	31	5	122	24.6	32.0	6.6	7.4	25.4	4.1
7944	CON	3	32	.	1	1	4	41	7.3	78.0	.	2.4	2.4	9.8
	VAC	8	29	5	.	1	.	43	18.6	67.4	11.6	.	2.3	.
7950	CON	12	54	8	5	11	7	97	12.4	55.7	8.2	5.2	11.3	7.2
	VAC	19	64	6	4	3	4	100	19.0	64.0	6.0	4.0	3.0	4.0
7951	CON	10	31	1	1	7	6	56	17.9	55.4	1.8	1.8	12.5	10.7
	VAC	17	33	2	1	4	1	58	29.3	56.9	3.4	1.7	6.9	1.7
7952	CON	15	31	.	4	6	3	59	25.4	52.5	.	6.8	10.2	5.1
	VAC	20	30	4	2	2	2	60	33.3	50.0	6.7	3.3	3.3	3.3
7953	CON	27	35	1	4	15	12	94	28.7	37.2	1.1	4.3	16.0	12.8
	VAC	29	37	5	1	9	11	92	31.5	40.2	5.4	1.1	9.8	12.0
All	CON	541	714	145	98	139	78	1715	31.5	41.6	8.5	5.7	8.1	4.5
	VAC	532	755	135	90	124	66	1702	31.3	44.4	7.9	5.3	7.3	3.9

Table 4.3 Least squares means and SEM for performance and health data following vaccination with an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.

	Control	Vaccinates	SEM	<i>P</i> -value
Enrollment Weight, lbs	749	746	3.5	0.52
HCW, lbs	777.4	774.7	11.8	0.14
ADG, lbs	2.97	2.94	0.05	0.15
Morbidity, %	7.3	8.5	1.3	0.20
Mortality, %	2.1	1.5	0.5	0.23

Table 4.4 Finishing ration formulation for Feedyard A used in the efficacy study investigating an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.

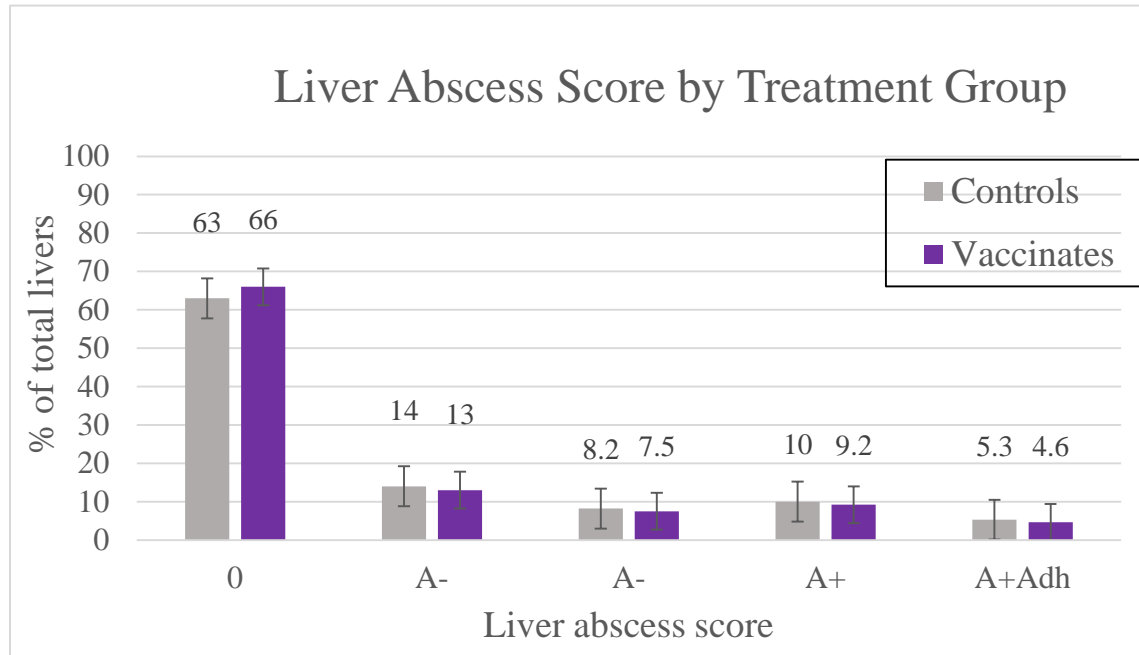
Ingredient	% of ration
Flaked Corn	69.0
Corn Oil	1.9
Corn Silage	18.1
Finisher	6
Micro-ingredients	1
STEEP	4

Table 4.5 Finishing ration formulation for Feedyard B used in the efficacy study investigating an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.

Ingredient	% of ration
Triticale Silage	4.4
Chopped Alfalfa Hay	3.1
Sunflower Screenings	4.4
Flaked Corn	66.3
CS/Cane Molasses (50% cane molasses, 50% low sulfur corn steep)	3.3
Dry Distillers Grain	12.1
Corn oil	1.8
Micro-ingredients	1.0
Finish Protein	3.6

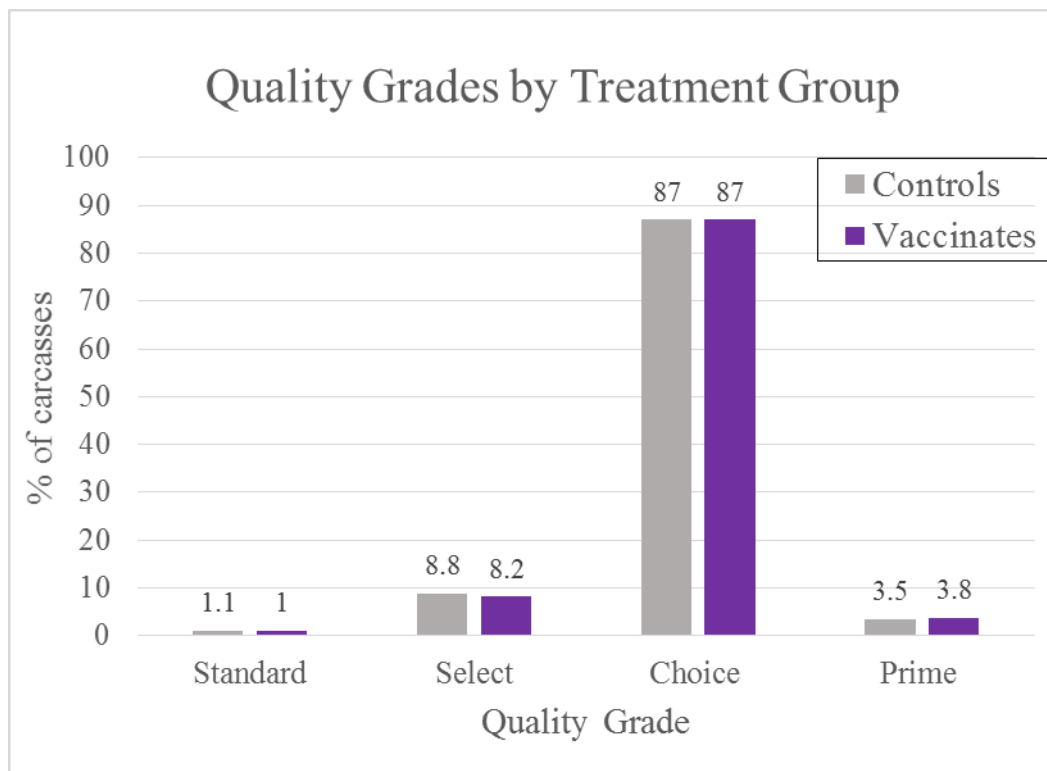
Figures

Figure 4.1 Liver abscess score by treatment group following vaccination with an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.



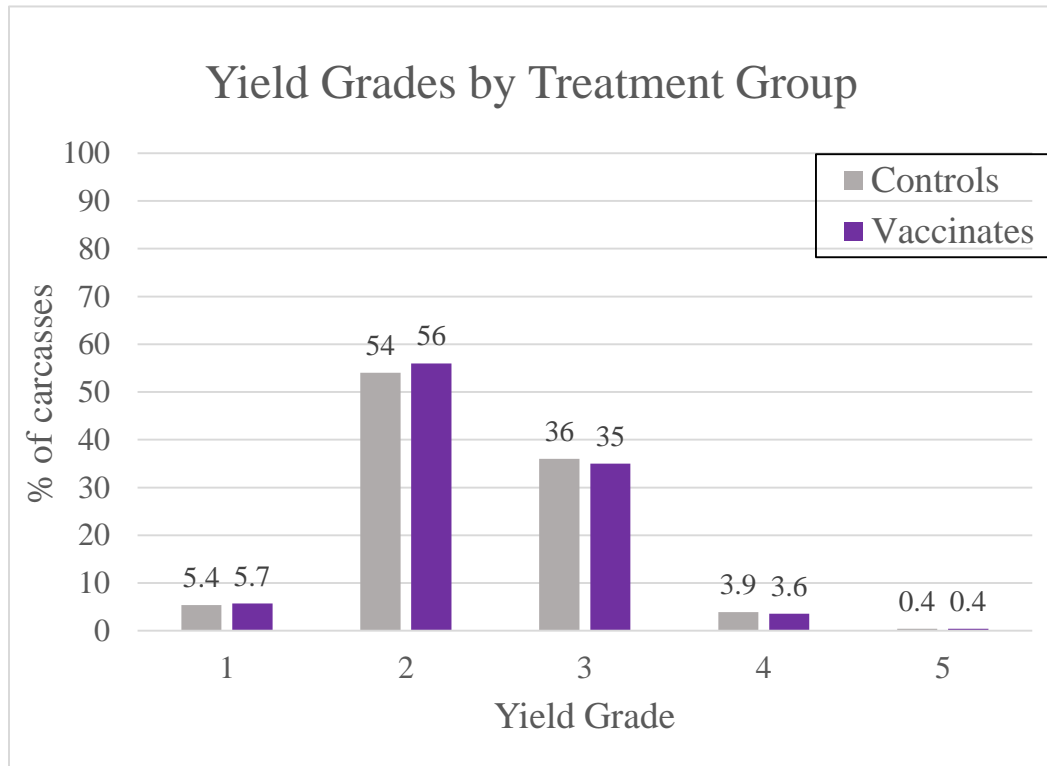
Liver abscess score did not differ by treatment ($P = 0.10$).

Figure 4.2 Quality grade data by treatment group following vaccination with an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.



Quality grade did not differ between treatment groups ($P = 0.46$).

Figure 4.3 Yield grade data by treatment group following vaccination with an autogenous vaccine containing an adjuvant assembled as a *Fusobacterium necrophorum* multivalent autogenous vaccine.



Yield grade did not differ between treatment groups ($P = 0.35$).

Chapter 5 - Research Implications

Liver abscesses are truly a problem found at the junction of animal health, nutrition, and husbandry. Moving forward, this is a critical problem for the United States cattle feeding industry to address. Results of the project outlined in chapter 2 of this dissertation imply that although liver abscesses can negatively impact quality grade, hot carcass weight, and feedlot performance, they do not cause decreased meat tenderness or eating quality within quality grade. This is good news for the beef industry as we have already seen an increase in the percent of carcasses with liver abscesses identified at the time of slaughter. This is partially due to the increasing consumer trend of desiring “natural” beef from those animals that have not been administered an antibiotic, in addition to the increased regulation surrounding antimicrobial use, particularly those deemed critically-important to human medicine like tylosin.

The autogenous vaccine against *F. necrophorum* sp. *necrophorum* researched here in chapters 3 and 4 was found to be safe to administer, however does not appear to reduce liver abscess prevalence in natural-fed cattle and therefore is not a beneficial product to use in this class of cattle. It has been speculated that the regional differences in liver abscess prevalence is attributable to differences in the strains of *F. necrophorum* present in the soil. If this does truly contribute to the regional differences, this needs to be taken into account and include strains of *F. necrophorum* that cause abscesses in the area where the vaccine is being used. Vaccine research should continue as a possible liver abscess prevention tool. Vaccines can be used in cattle enrolled in “natural” programs, which anecdotally have higher liver abscess prevalence than conventionally-fed cattle. If vaccines were competitively priced and as efficacious as tylosin, it would most likely be used in much of the fed cattle industry. This would be advantageous from a ration handling standpoint in that it would remove a daily feed additive (tylosin). Previous

vaccines have been found to be effective at decreasing prevalence and severity in groups of cattle with low prevalence of liver abscesses. As we continue to learn more about liver abscess formation, bacterial virulence factors, animal pathophysiology, and vaccine development, it is possible that a vaccine will be developed that can more effectively prevent and decrease severity of liver abscesses in feedlot cattle.